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Photocarcinogenesis Study of Glycolic Acid and Salicylic Acid in SKH-1 Mice

NTP Technical Report 524

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OUTLINE

- Background
- Study methods
  - Study design
  - Test Articles
  - Doses of Light
- Significant findings
  - Time to First Tumor
  - Pathology findings
- Conclusions
- Tumor multiplicity
- Acknowledgments

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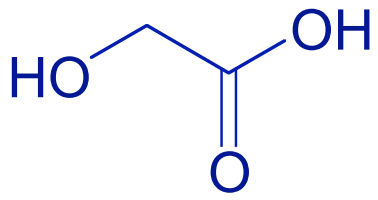
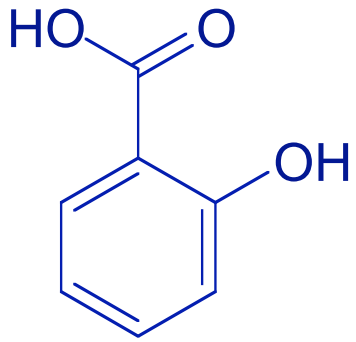
BACKGROUND

- Uses of AHA and BHA
  - Topical creams
  - Higher concentrations as chemical peels
  - Lower concentration as cleanser, moisturizer, etc
  - Correction of photoaging
- Structures of glycolic and salicylic acid



glycolic acid



salicylic acid

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BACKGROUND

- FDA nomination
  - Use on photo-exposed skin; increased sensitivity to sun
  - Removal of stratum corneum
  - Increased cell proliferation

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PURPOSE OF STUDY

Test effect of topical application of cream containing AHA (glycolic acid) or BHA (salicylic acid) on UVB induced skin cancer development (photococarcinogenesis)

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STUDY DESIGN

Test Animal

Crl: SKH-1 (hr^{-}/hr^{-}) hairless mice

males and females

18 or 36 mice/group

8 weeks of age at start of treatment

Test Article

Topically applied (AM)

~2 mg/cm^2 cream, volumetric delivery

<30 sec application

base of tail to base of neck; to tangent on flanks

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STUDY DESIGN (page 2)

Irradiation with light

0, 0.3, 0.6 and 0.9 $MED^{instrumental}$ (sub-erythemic)

Treatment (PM) 5 days/week

Observations

Standard clinical observations recorded

Weekly tumor measurements (<1, 1-2, 2-3, 3-5, 5-7, 7-10, >10 mm)

40 weeks, test chemical and light

12 weeks, observation

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TEST GROUPS

Cream Application	No Light	0.3 MED[i]	0.6 MED[i]	0.9 MED[i]
None	36*	36	36	36
Control cream, pH 3.5	18	18	18	-
4% Glycolic acid (GA), pH 3.5	18	18	18	-
10% GA, pH 3.5	18	18	18	-
2% Salicylic acid (SA), pH 4	18	18	18	-
4% SA, pH 4	18	18	18	-

**Number of males and females per group*

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CONTENT OF CONTROL CREAM

Water, 70.02%
Glycerin (96%), 3.25%
Keltrol T solution (2%), 8.00%
Veegum Ultra, 1.20%
Cetearyl alcohol, 2.50%
Eutanol G, 4.00%
Dimethicone DC200-100, 0.80%
Lipomulse 165, 2.40%
Brij 721 (Steareth-21), 2.40%
Lipowax D, 4.00%
Germaben II, 1.00%
Phosphoric acid (8.5%), 0.43%
pH 3.5

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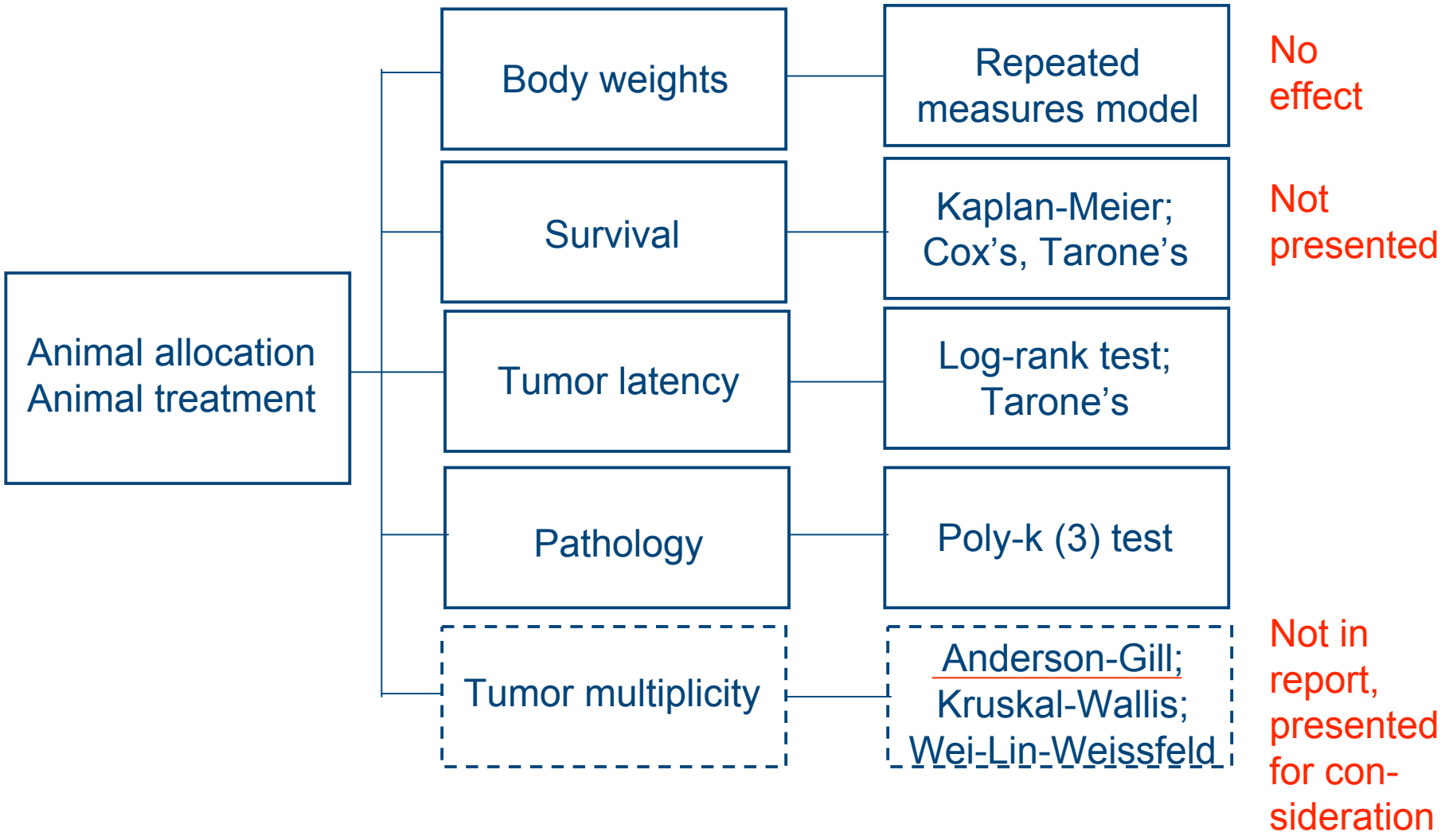
DATA FROM STUDY, STATISTICAL ANALYSES



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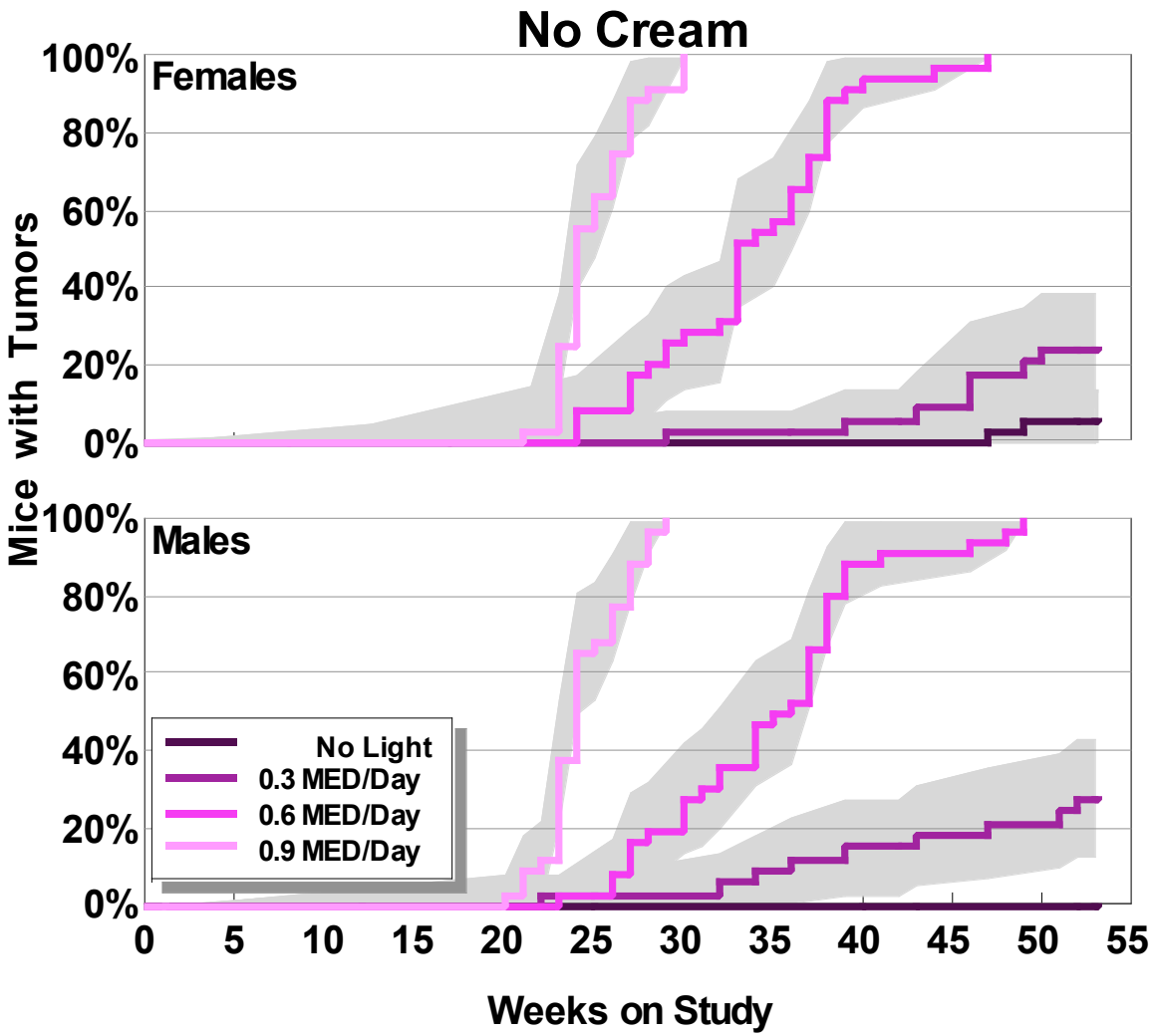
SURVIVAL

Mouse is removed from study if -

- Moribund
- Health status inconsistent with continuation
- Tumor reached 10 mm
- Tumors merged eliminating tumor individuality
- Skin condition inconsistent with continuation on study

TIME TO FIRST TUMOR ≥1 mm: no cream



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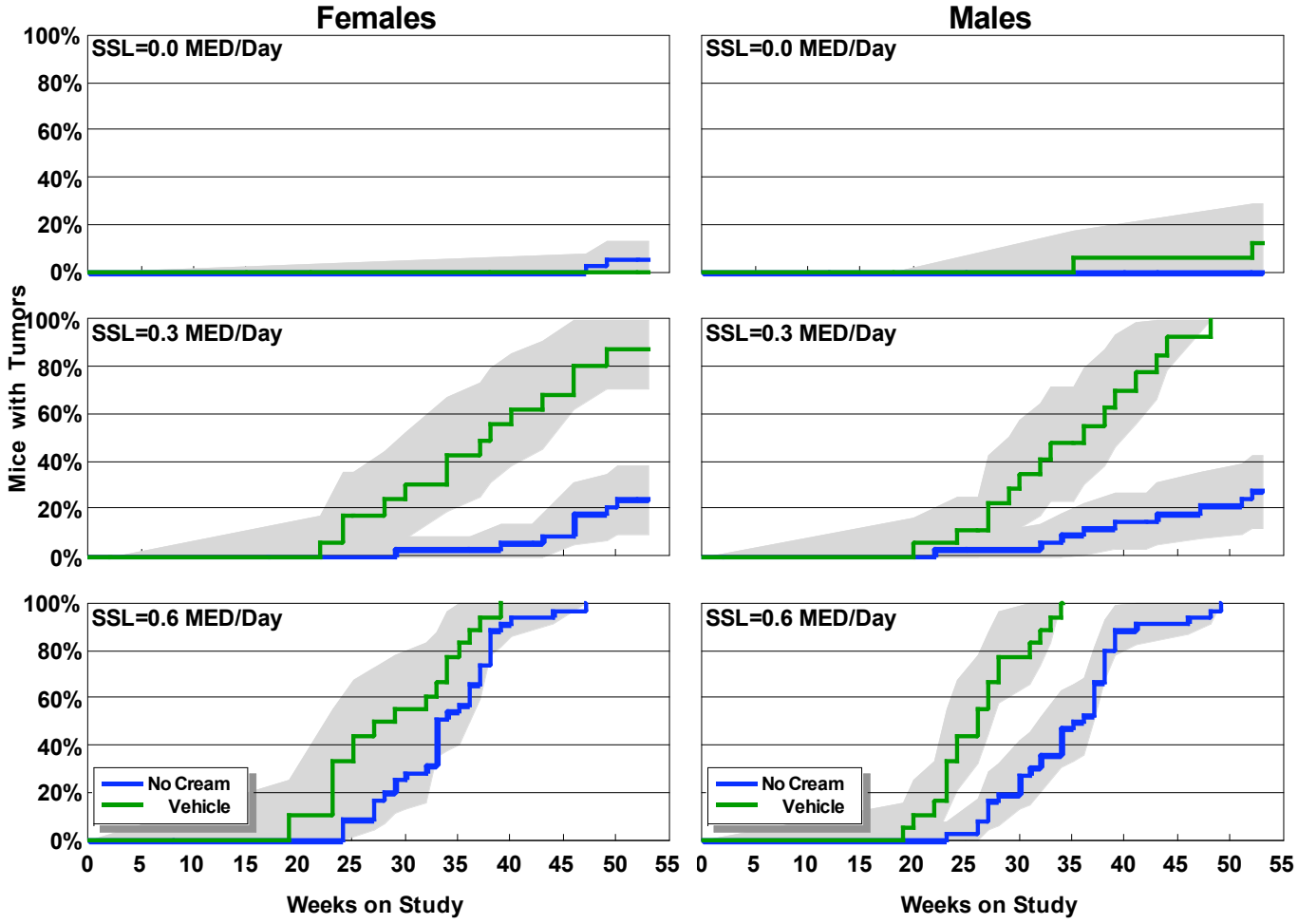
TIME TO FIRST TUMOR ≥1 mm: no cream vs. control cream



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TIME TO FIRST TUMOR ≥1 mm: no cream vs. control cream

Group mean time to tumor (weeks)

	None	Control Cream	
Female			
0.3 MED/d	48.5 ± 0.7	37.2 ± 2.3	*p=0.001*
0.6 MED/d	33.7 ± 0.9	28.7 ± 1.5	*p=0.005*
Male			
0.3 MED/d	48.6 ± 1.3	34.9 ± 2.0	*p=0.001*
0.6 MED/d	34.8 ± 1.0	26.1 ± 1.0	*p=0.001*

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CONCLUSIONS (1)

- Application of control cream decreased the group mean time to first tumor (≥ 1 mm),
- As a result, glycolic acid and salicylic acid groups should be compared to control cream results.

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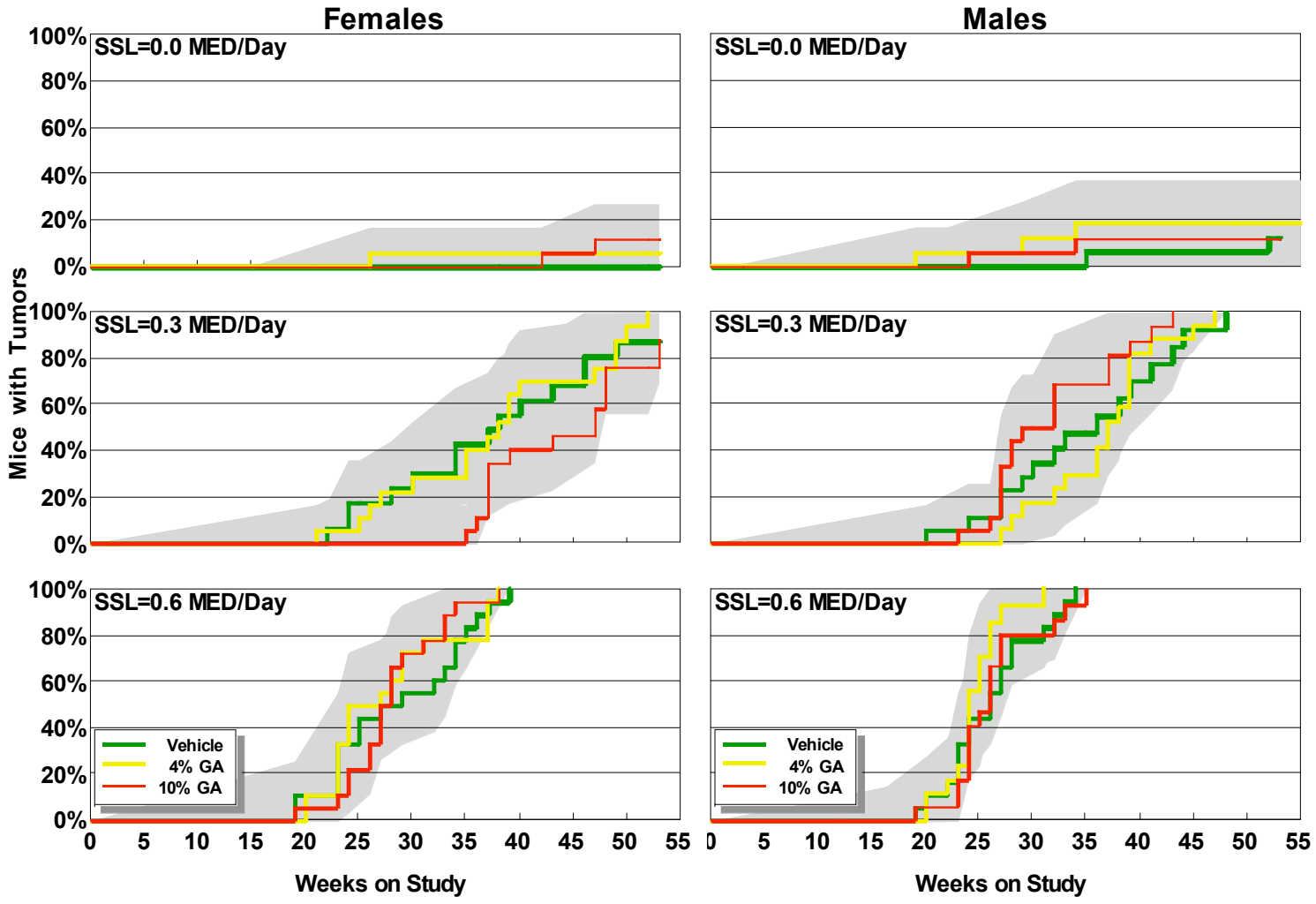
TIME TO FIRST TUMOR ≥1 mm: cream ± glycolic acid



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TIME TO FIRST TUMOR >1 mm: cream ± glycolic acid

Group mean time to tumor (weeks)

	Control Cream	4% GA	10% GA
Female			
0.3 MED/d	**37.2 ± 2.3** ***ns (trend)***	**37.8 ± 2.3** ***ns***	**44.2 ± 1.7** ***ns***
0.6 MED/d	**28.7 ± 1.5** ***ns***	**27.6 ± 1.4** ***ns***	**28.1 ± 1.1** ***ns***
Male			
0.3 MED/d	**34.9 ± 2.0** ***p=0.034***	**36.6 ± 1.3** ***ns***	**31.8 ± 1.4** ***ns***
0.6 MED/d	**26.1 ± 1.0** ***ns***	**24.5 ± 0.7** ***ns***	**26.3 ± 1.0** ***ns***

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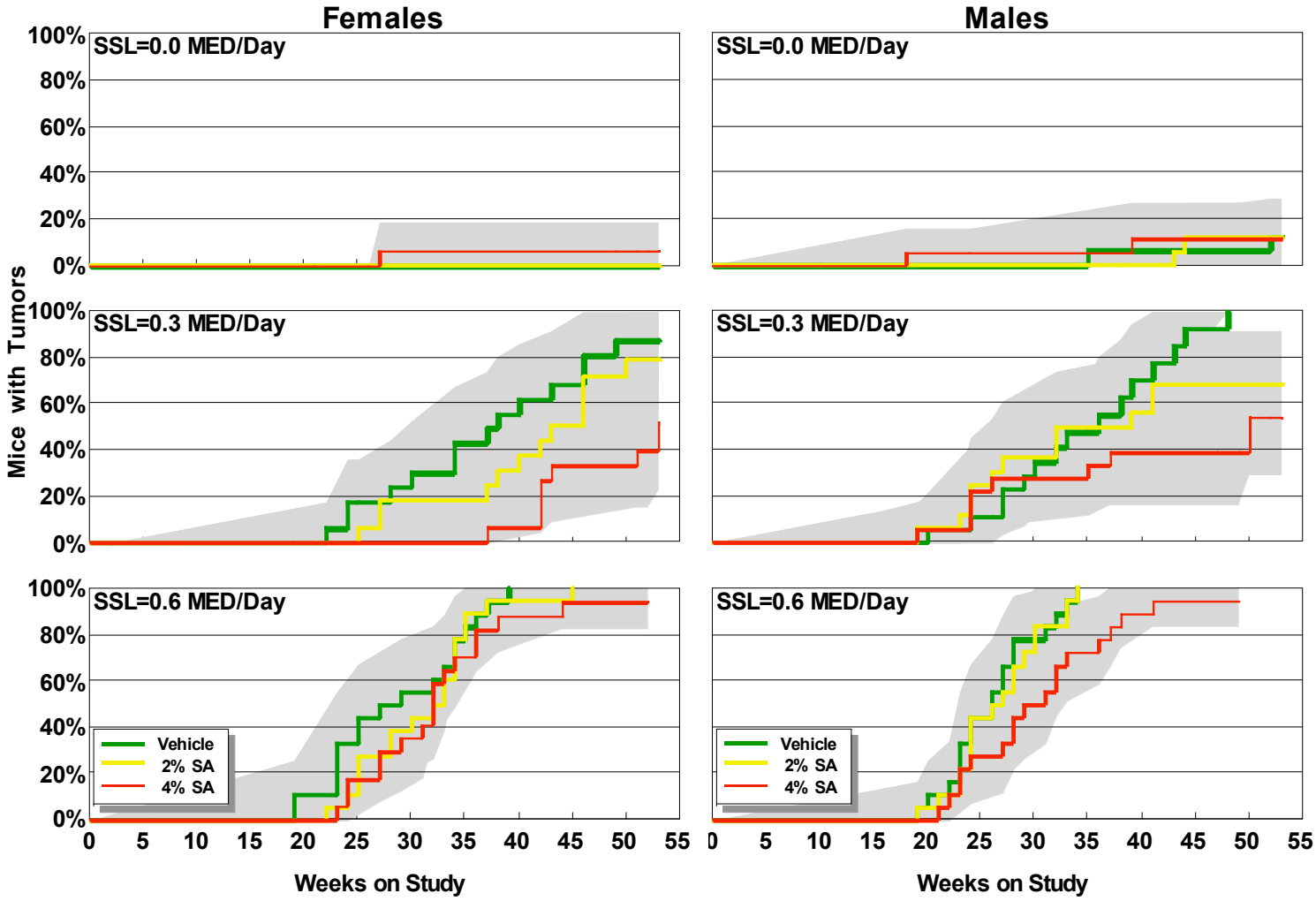
TIME TO FIRST TUMOR ≥1 mm: cream ± salicylic acid



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TIME TO FIRST TUMOR ≥1 mm: cream ± salicylic acid

Group mean time to tumor (weeks)

	Control Cream	2% SA	4% SA
Female			
0.3 MED/d	37.2 ± 2.3 *p=0.001N*	41.5 ± 2.2 *ns*	48.9 ± 1.6 *p=0.002N*
0.6 MED/d	28.7 ± 1.5 *ns*	31.1 ± 1.3 *ns*	32.1 ± 1.5 *ns*
Male			
0.3 MED/d	34.9 ± 2.0 *p=0.008N*	33.4 ± 2.1 *ns*	41.1 ± 3.0 *p=0.003N*
0.6 MED/d	26.1 ± 1.0 *p=0.005N*	26.7 ± 1.0 *ns*	30.3 ± 1.5 *p=0.008N*

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CONCLUSIONS (2)

Compared to control cream,

- Glycolic acid did not have consistent effect on skin tumor (≥ 1 mm) development.

- Salicylic acid increased mean time to first tumor (≥ 1 mm) (*i.e.* protective) at 0.3 MED/d in females, and 0.3 and 0.6 MED/d in males.

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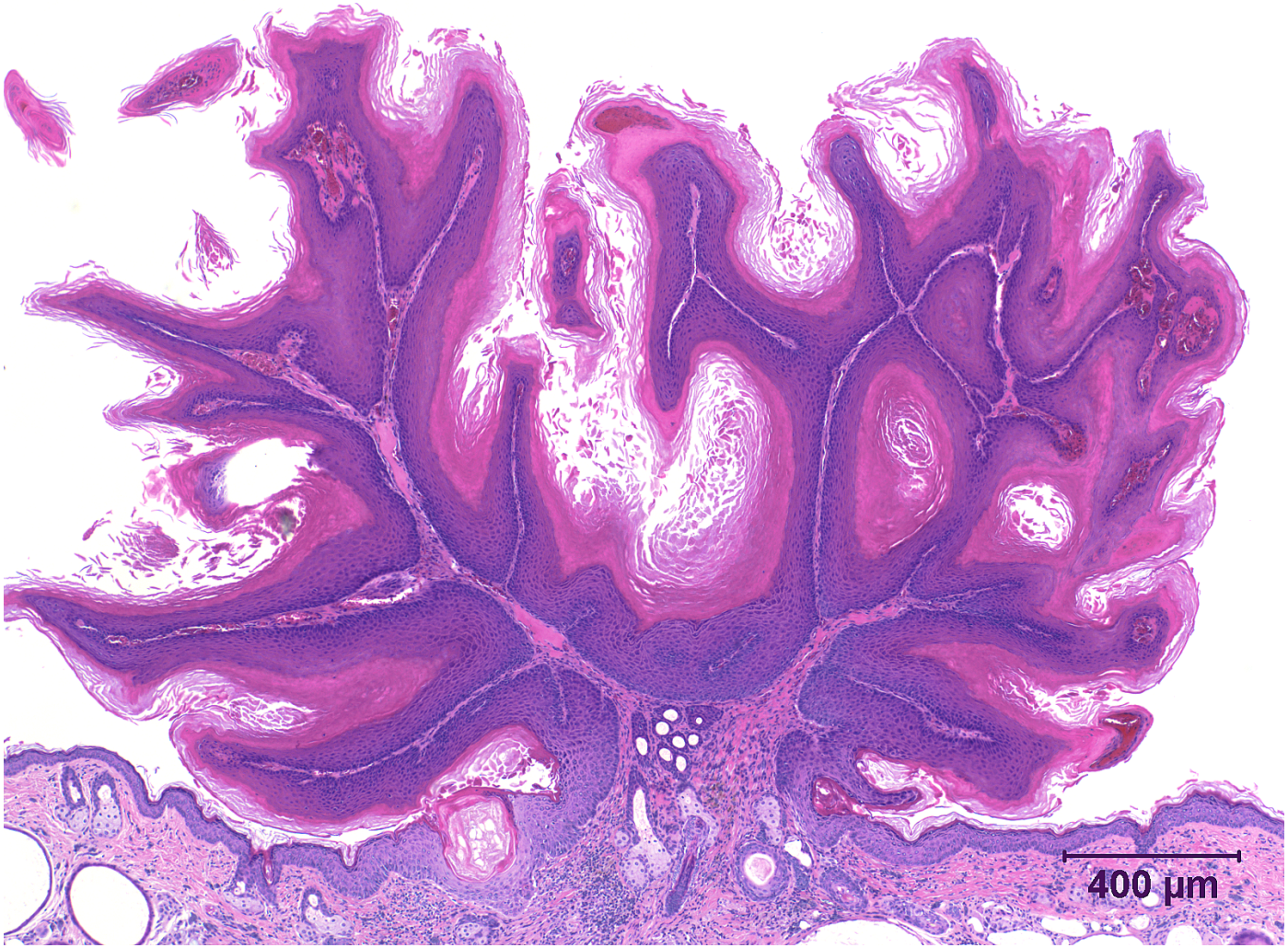
SQUAMOUS CELL PAPILLOMA



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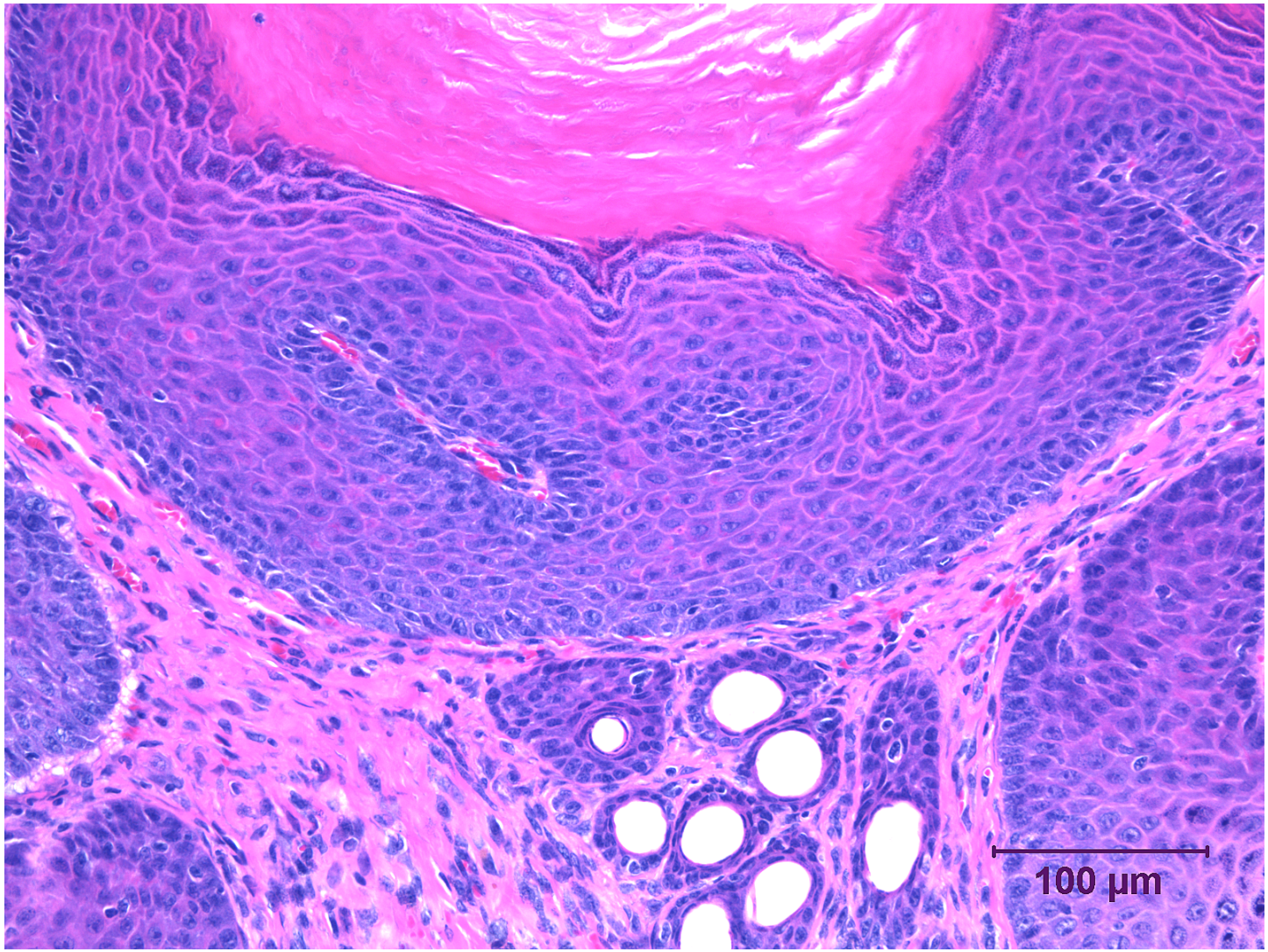
SQUAMOUS CELL PAPILLOMA



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CARCINOMA *IN SITU*

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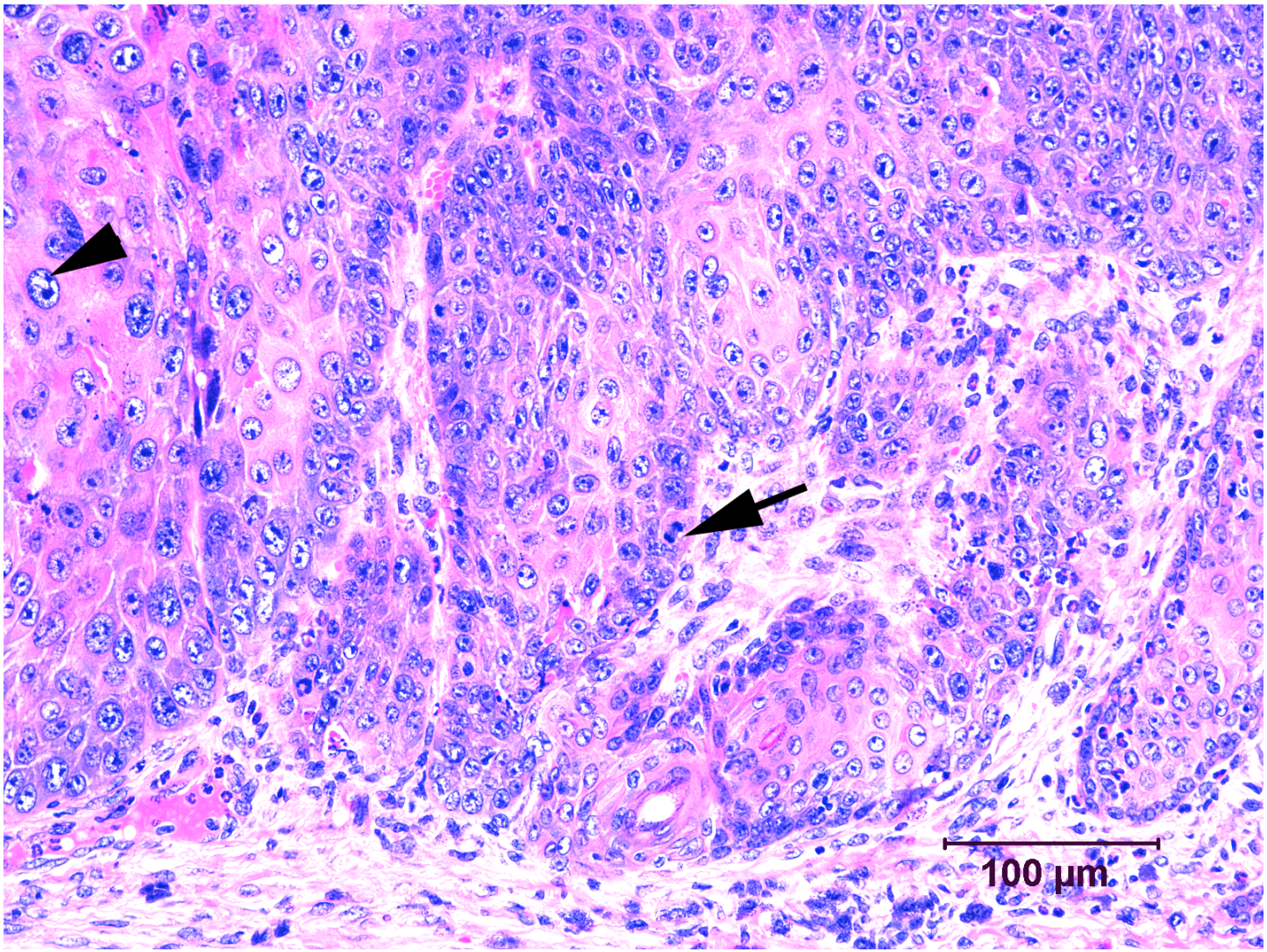
CARCINOMA *IN SITU*



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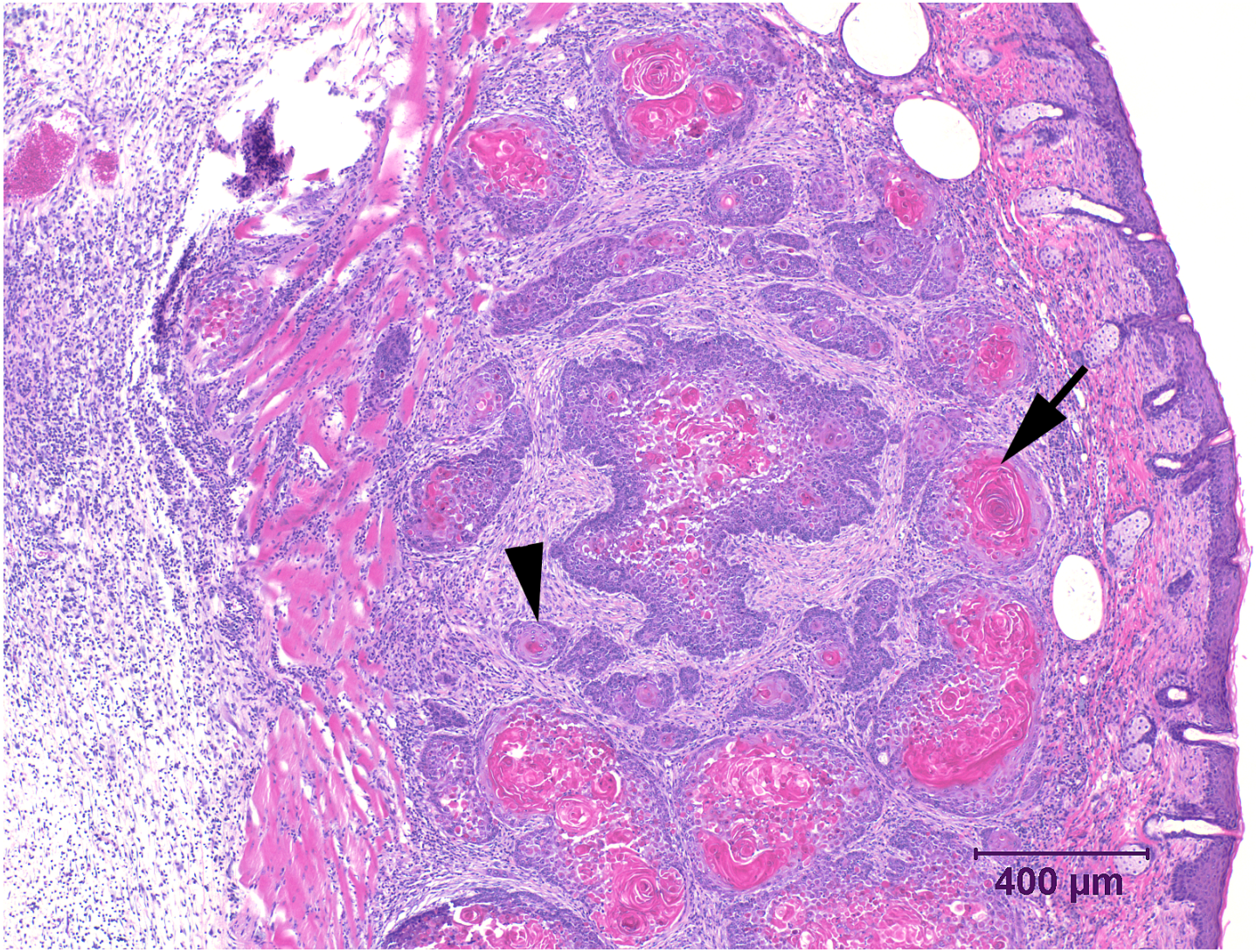
SQUAMOUS CELL CARCINOMA



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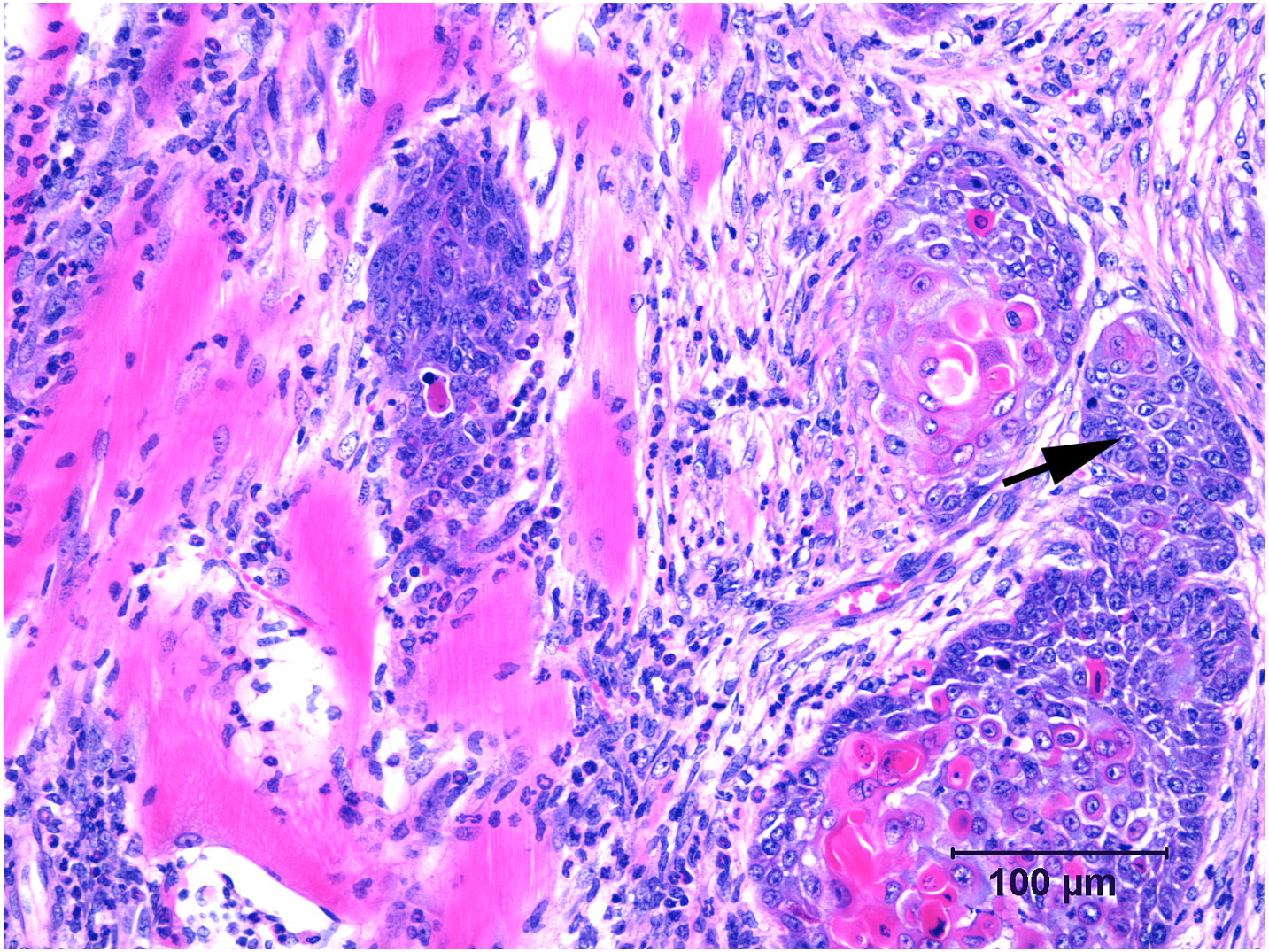
SQUAMOUS CELL CARCINOMA



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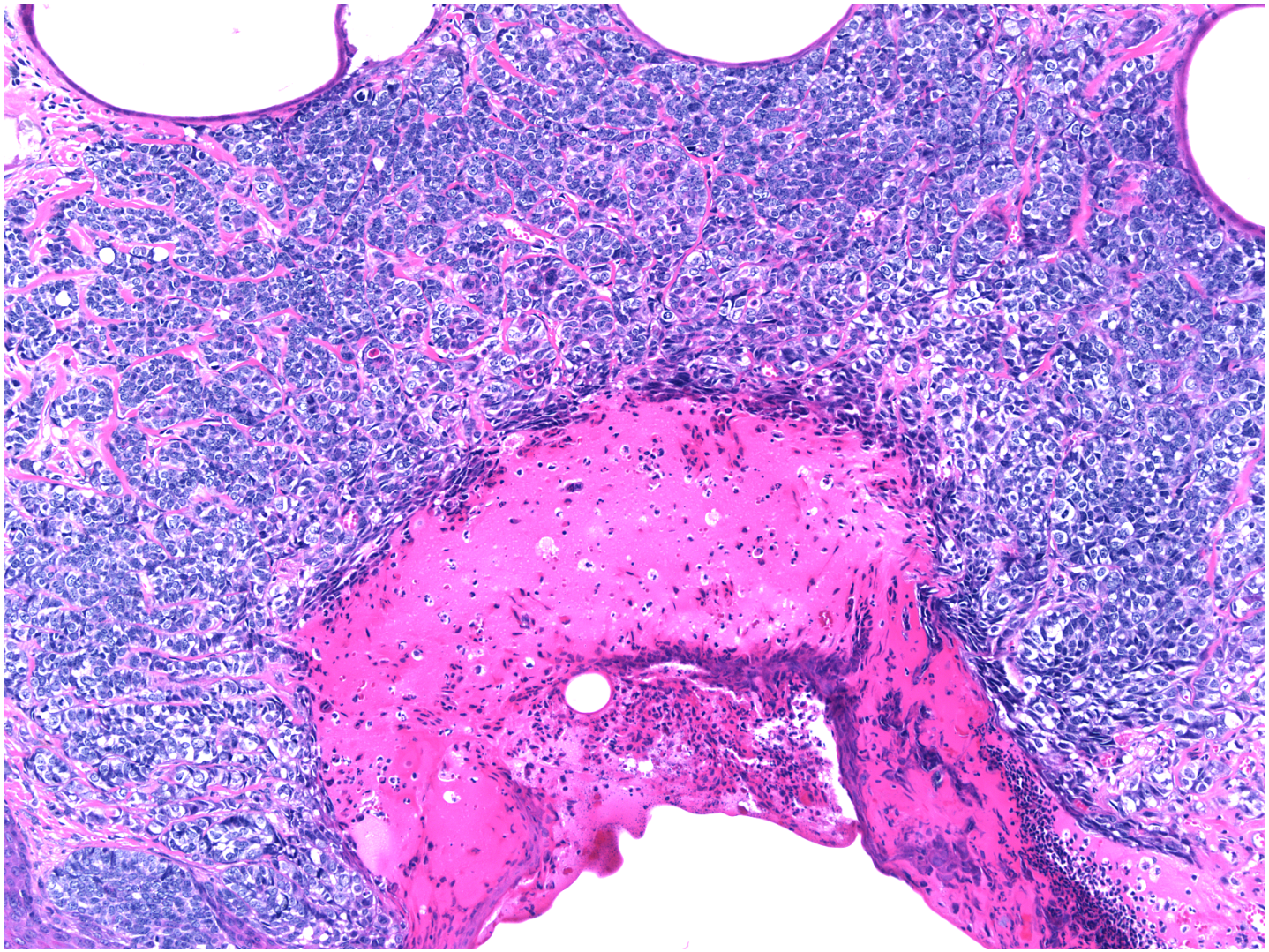
BASAL CELL CARCINOMA



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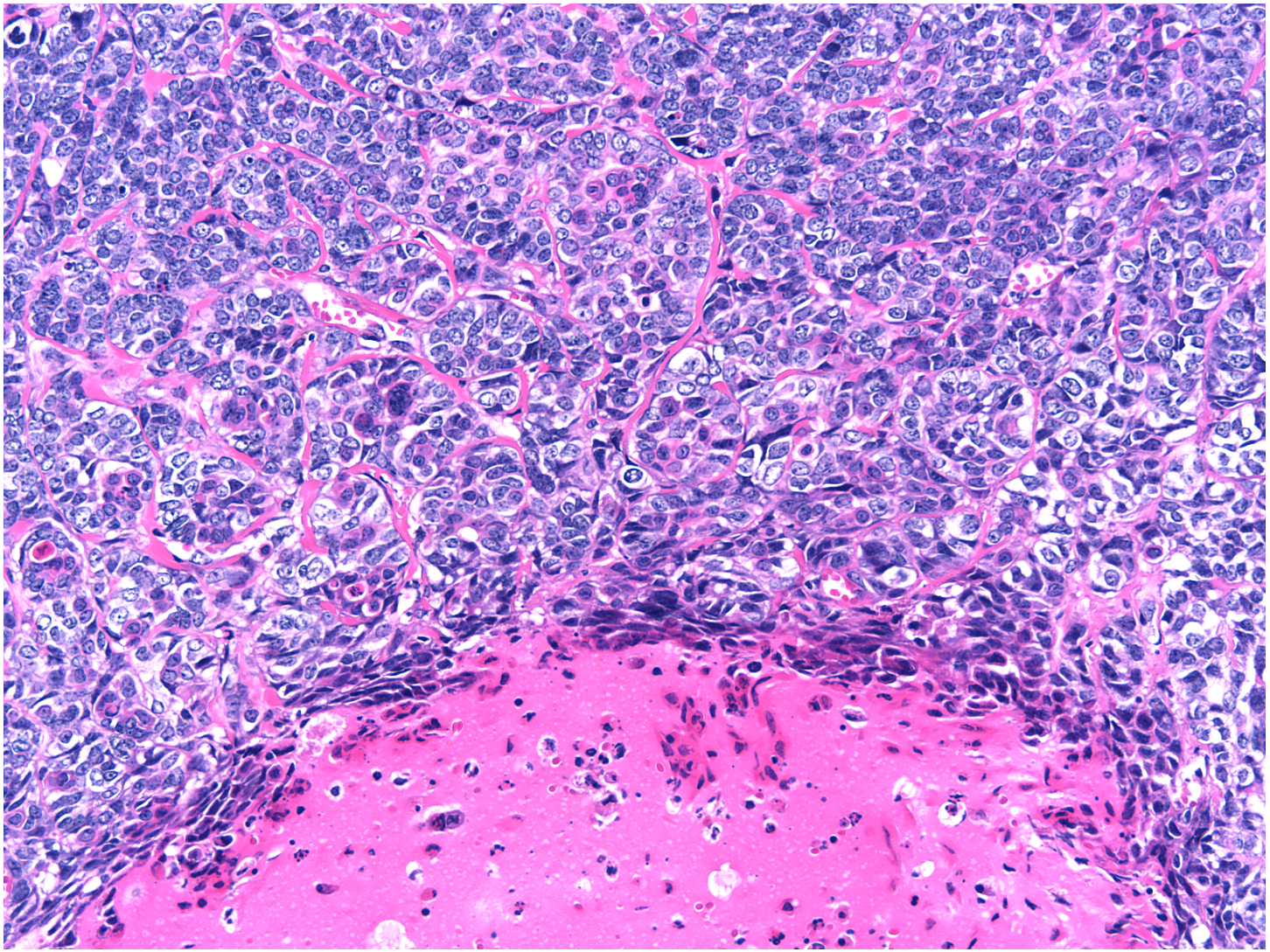
BASAL CELL CARCINOMA



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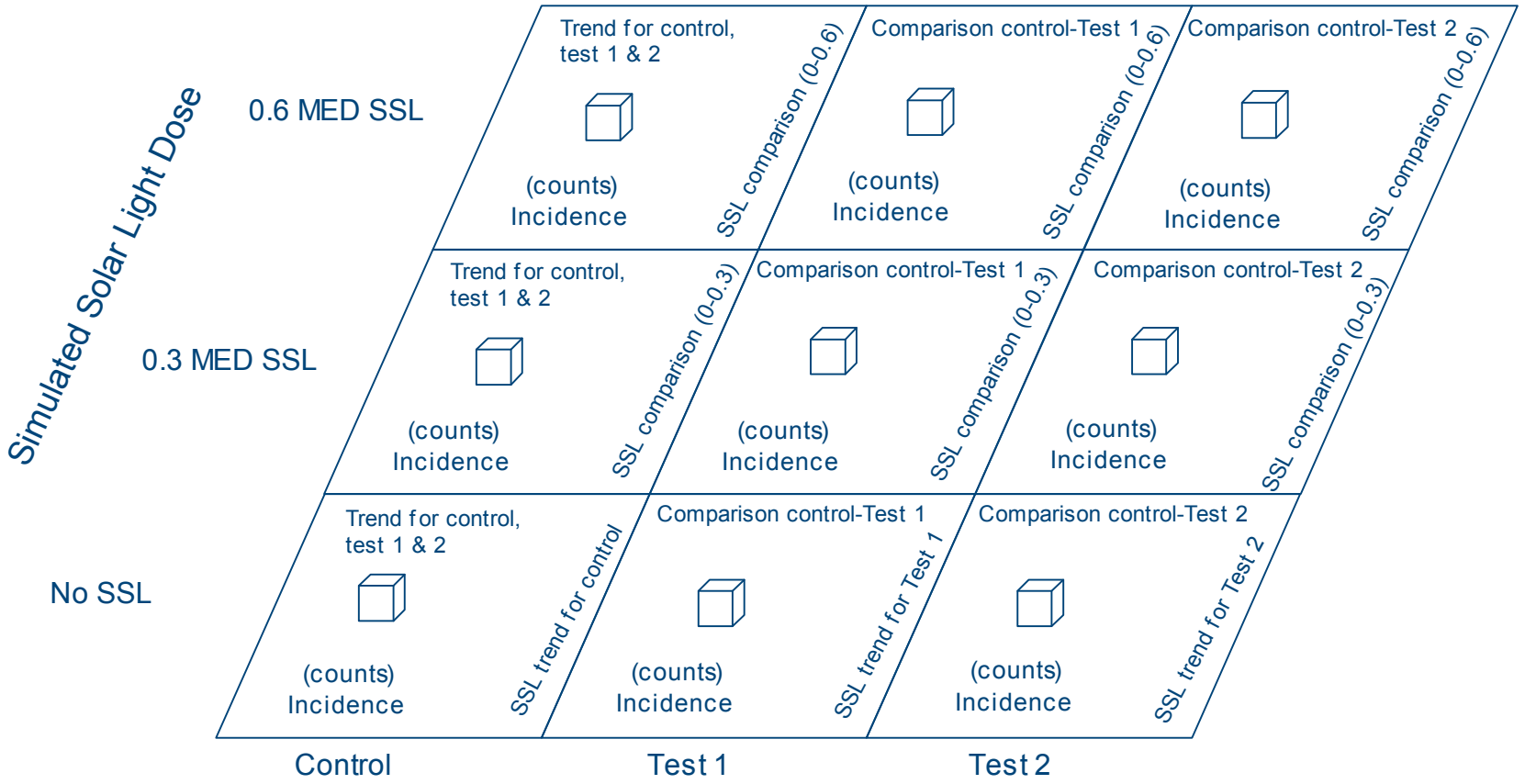
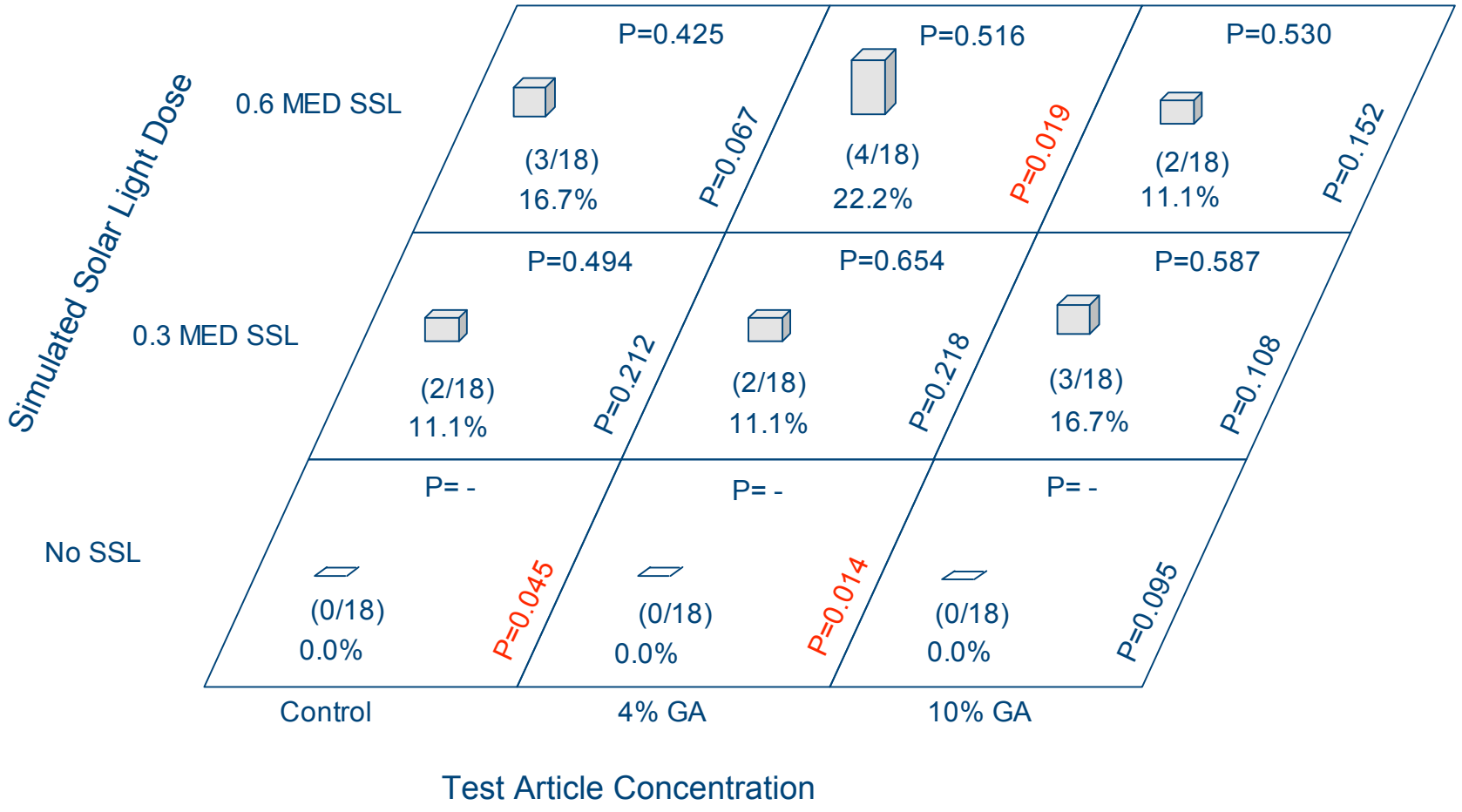
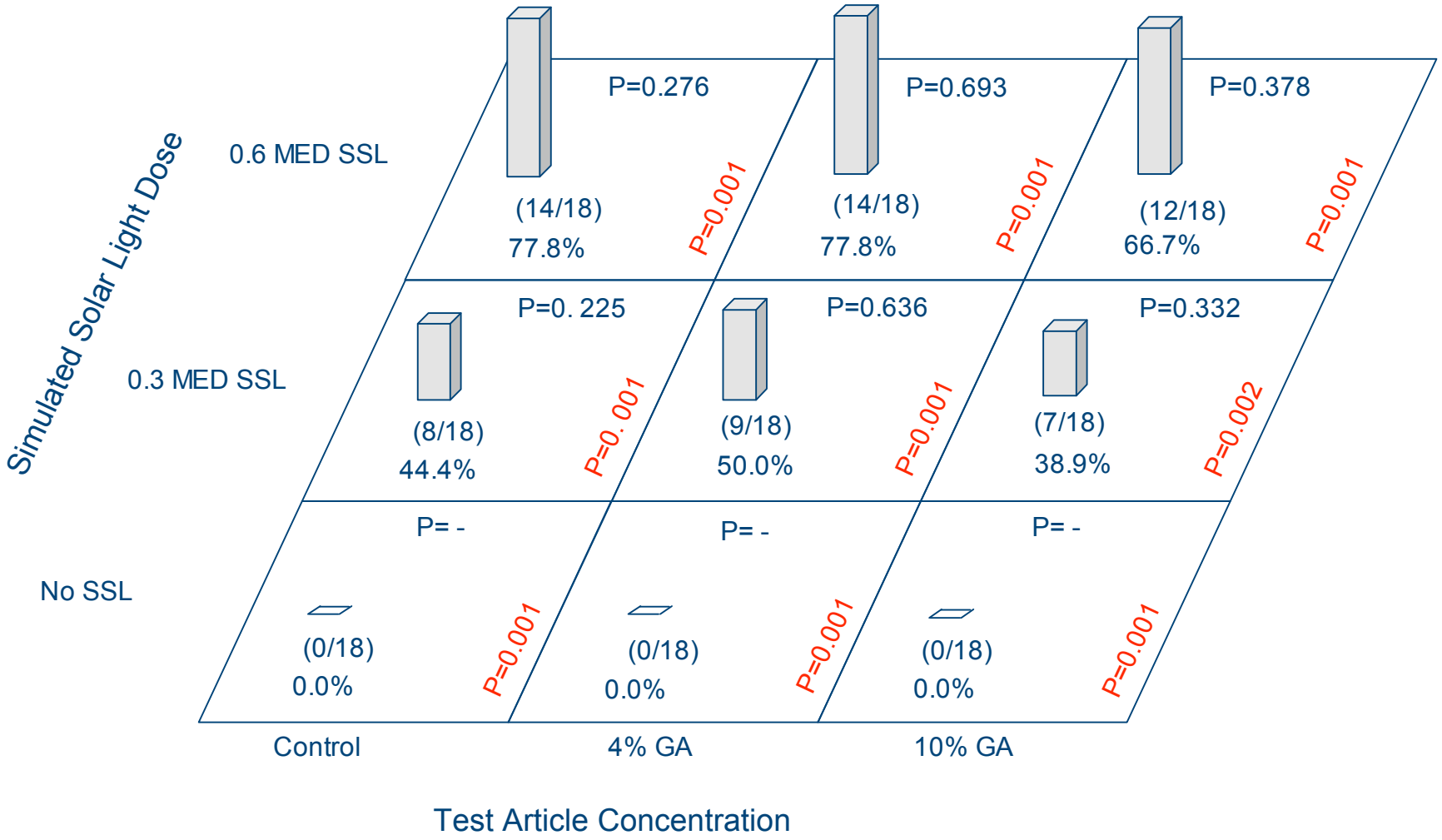
EXAMPLE OF BLOCK CHART



FEMALE MICE, GLYCOLIC ACID; SQUAMOUS CELL PAPILLOMA



FEMALE MICE, GLYCOLIC ACID; CARCINOMA *IN SITU*



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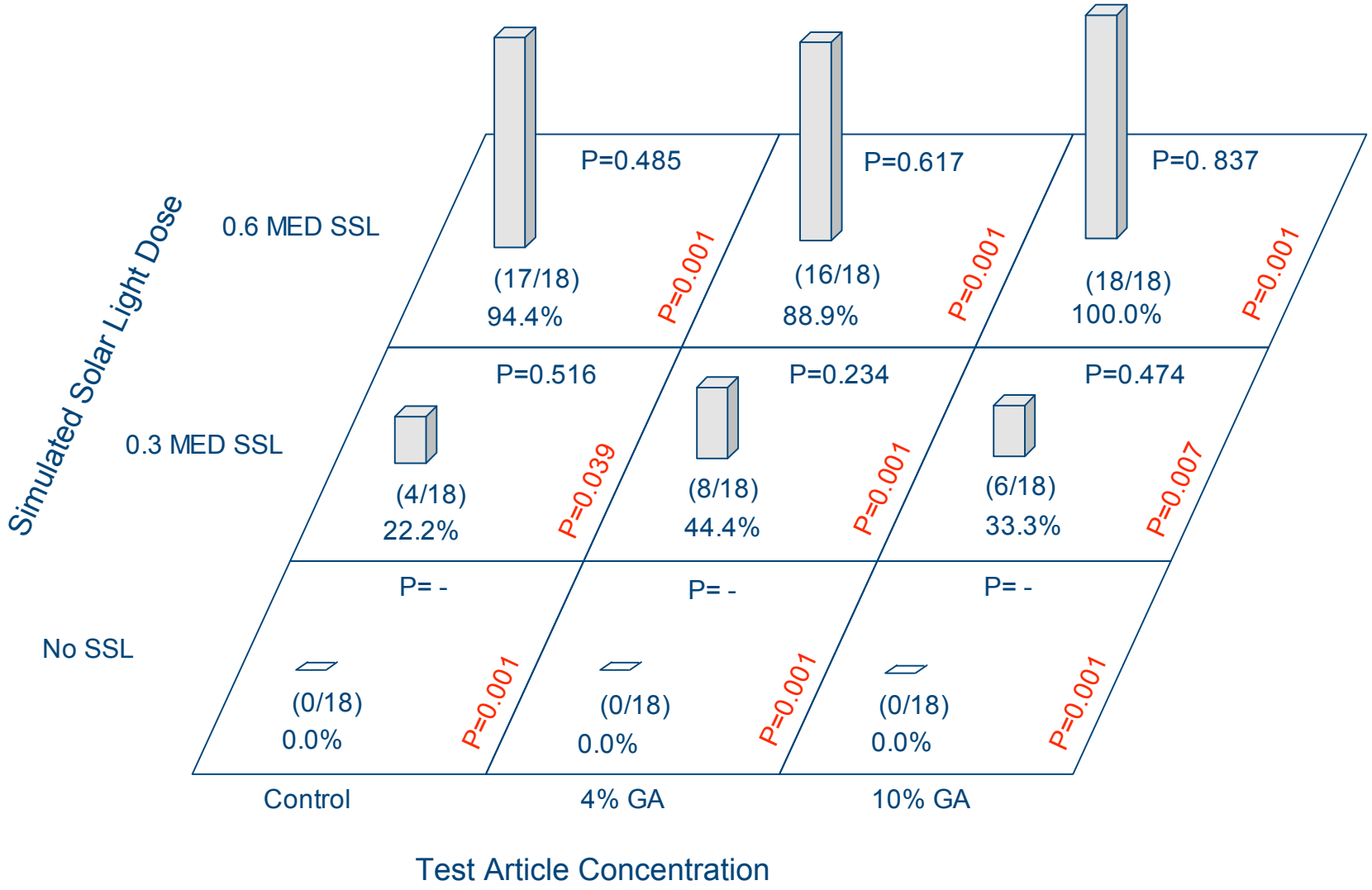
FEMALE MICE, GLYCOLIC ACID; SQUAMOUS CELL CARCINOMA



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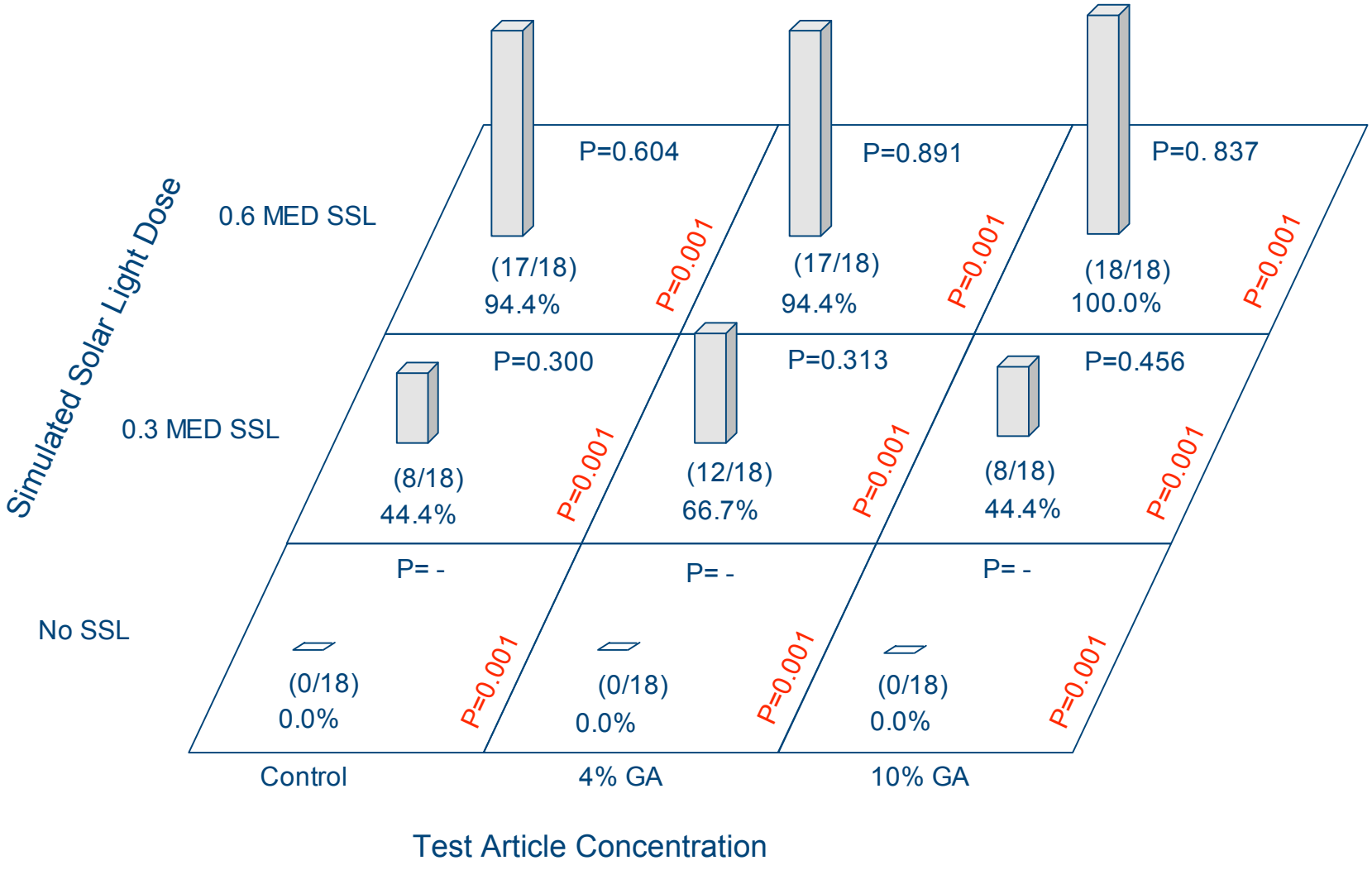
FEMALE MICE, GLYCOLIC ACID; ALL SKIN CANCERS



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CONCLUSIONS, PATHOLOGY (1)

- Glycolic acid did not affect tumorigenesis of SSL in female mice.

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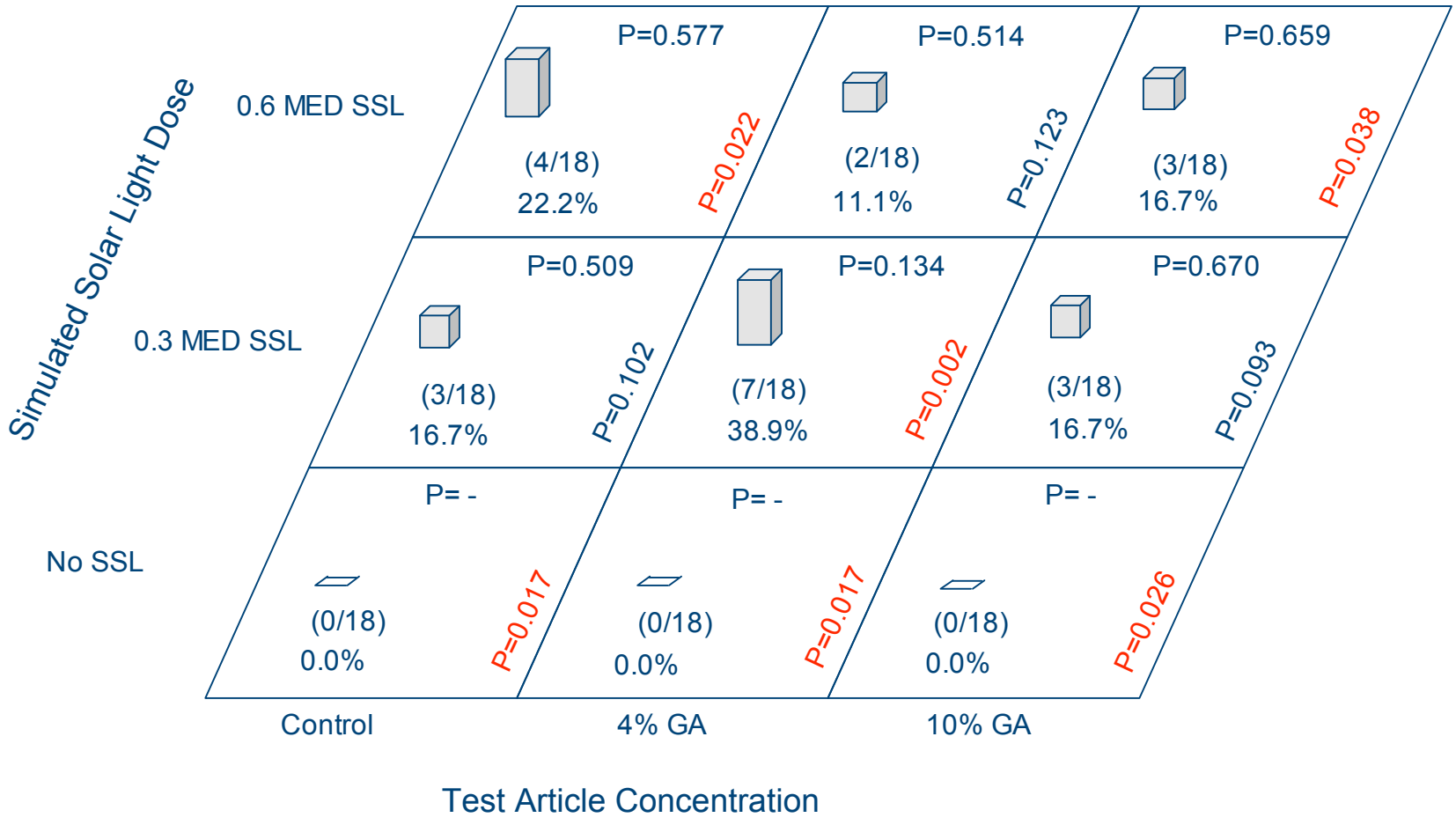
MALE MICE, GLYCOLIC ACID; SQUAMOUS CELL PAPILLOMA



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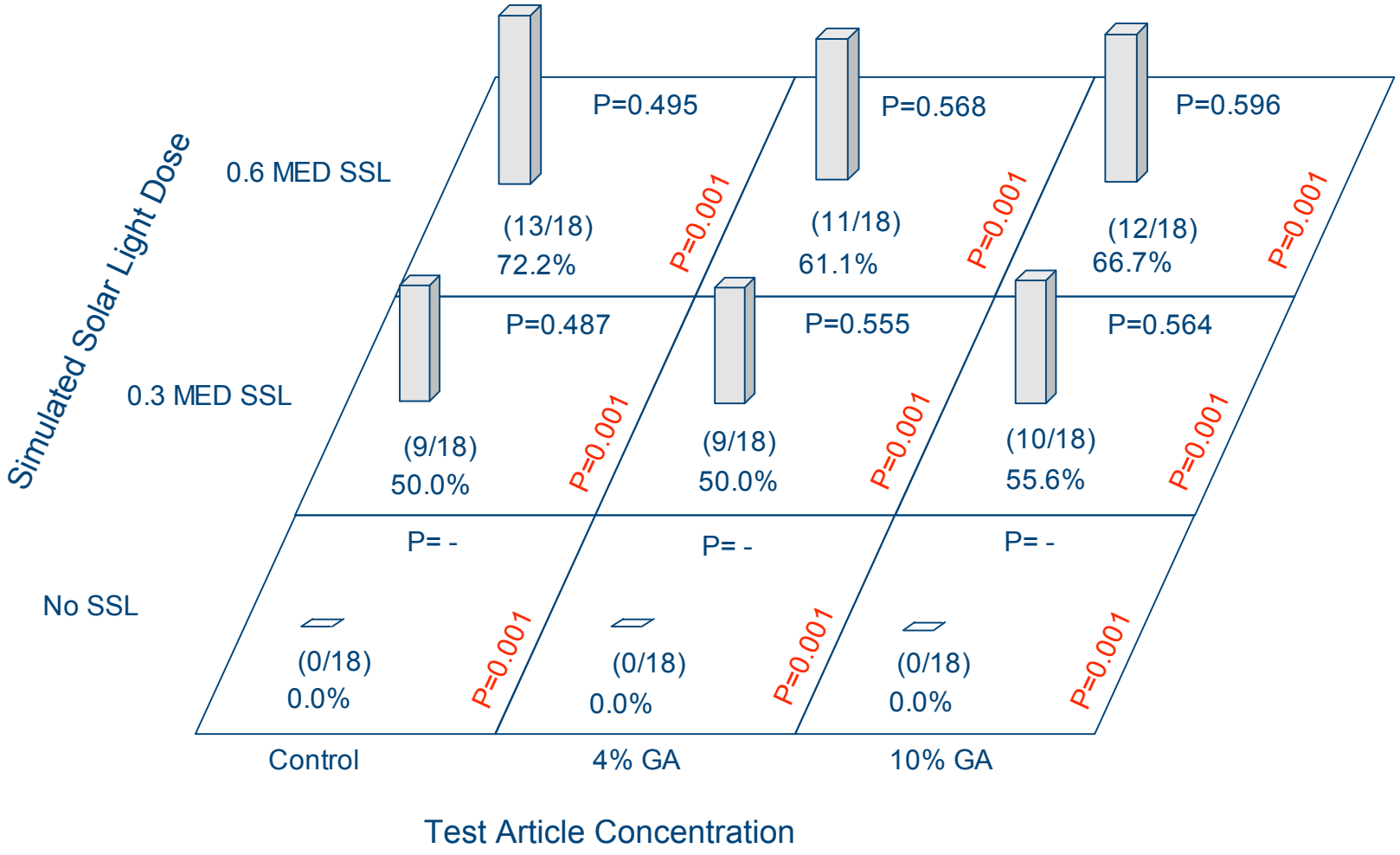
MALE MICE, GLYCOLIC ACID; CARCINOMA *IN SITU*



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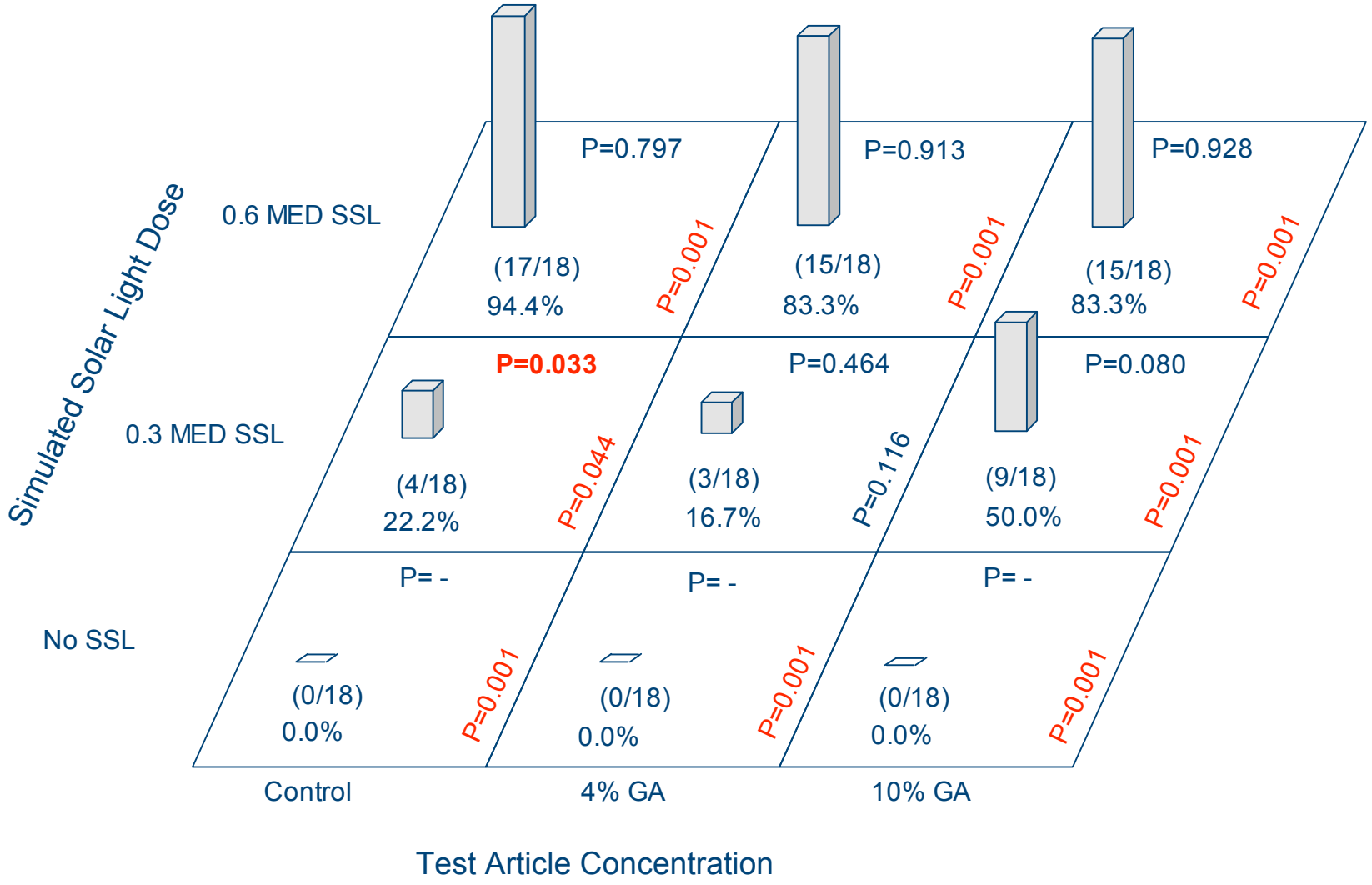
MALE MICE, GLYCOLIC ACID; SQUAMOUS CELL CARCINOMA



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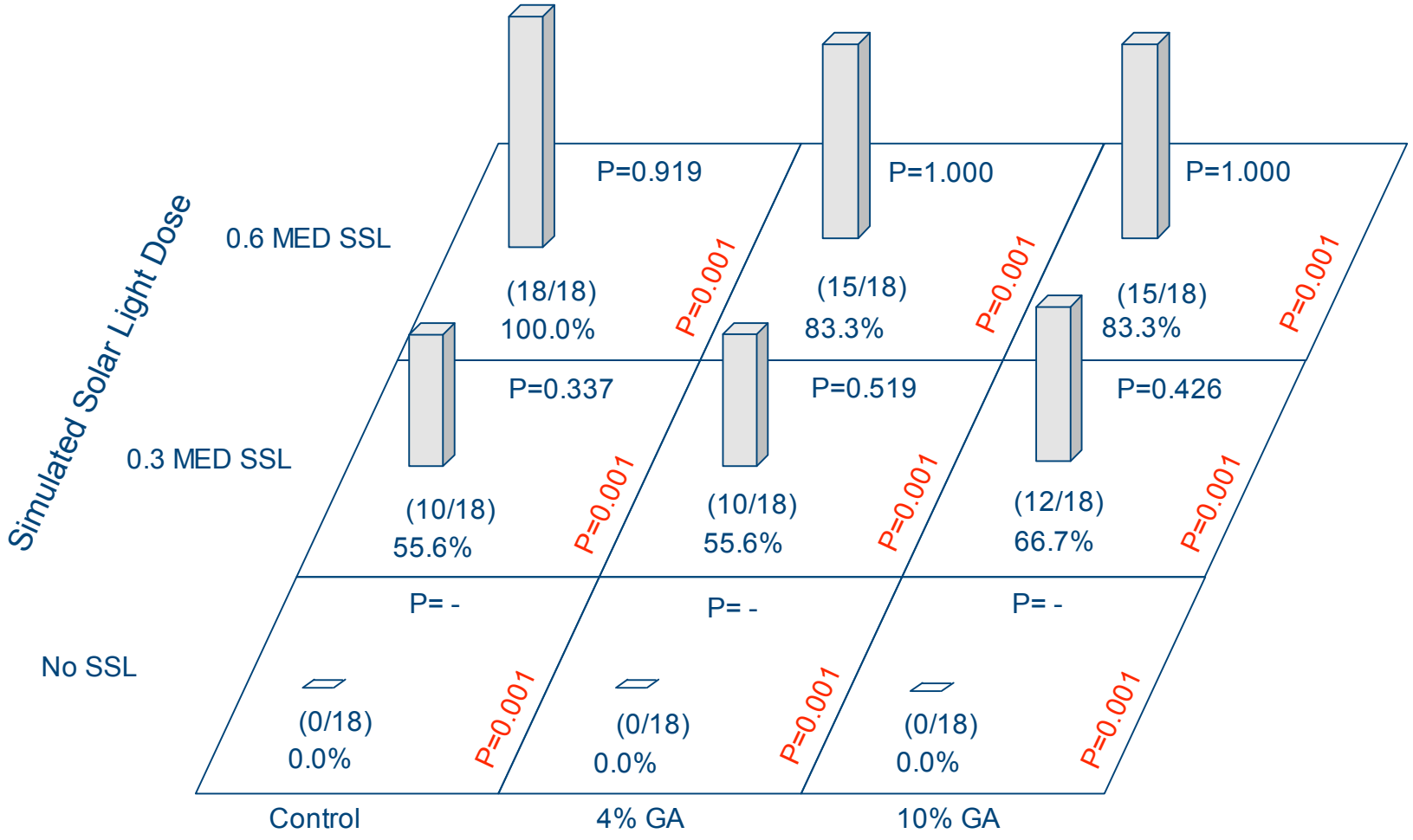
MALE MICE, GLYCOLIC ACID; ALL SKIN CANCERS



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CONCLUSIONS, PATHOLOGY (2)

- Glycolic acid did not affect tumorigenesis of SSL in female mice,

- Glycolic acid did not consistently affect tumorigenesis of SSL in male mice.

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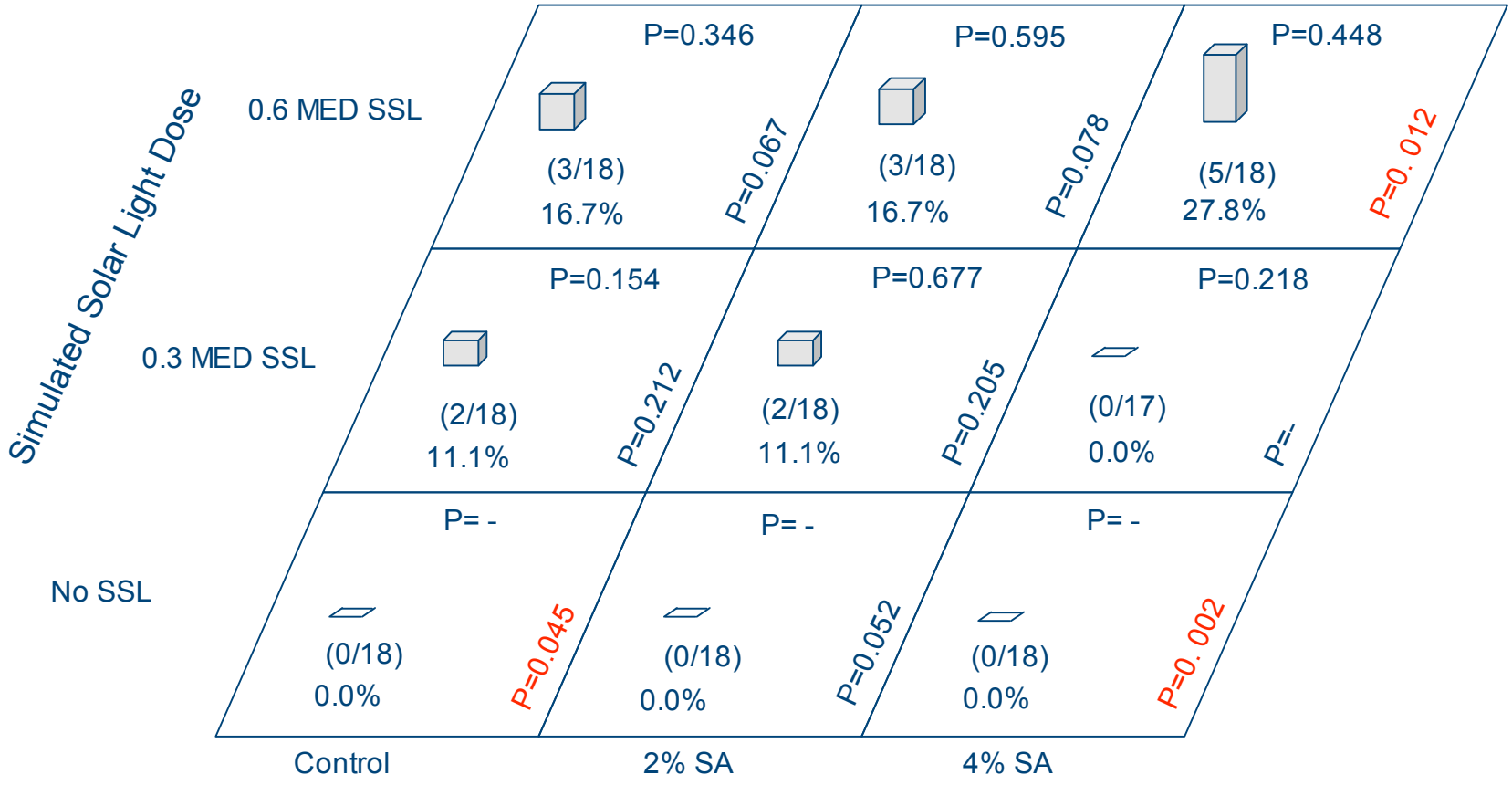
FEMALE MICE, SALICYCLIC ACID; SQUAMOUS CELL PAPILLOMAS



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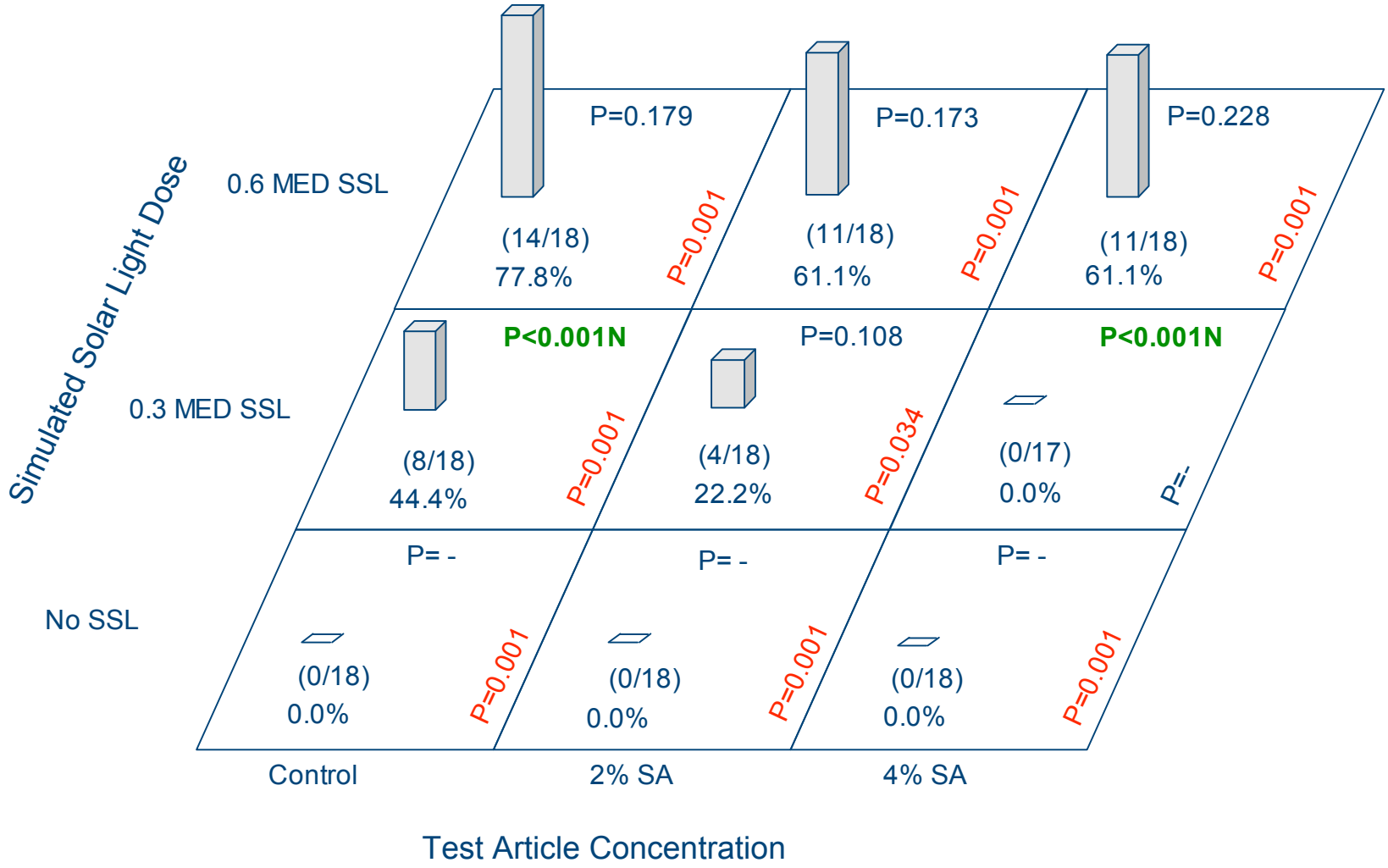
FEMALE MICE, SALICYLIC ACID; CARCINOMA *IN SITU*



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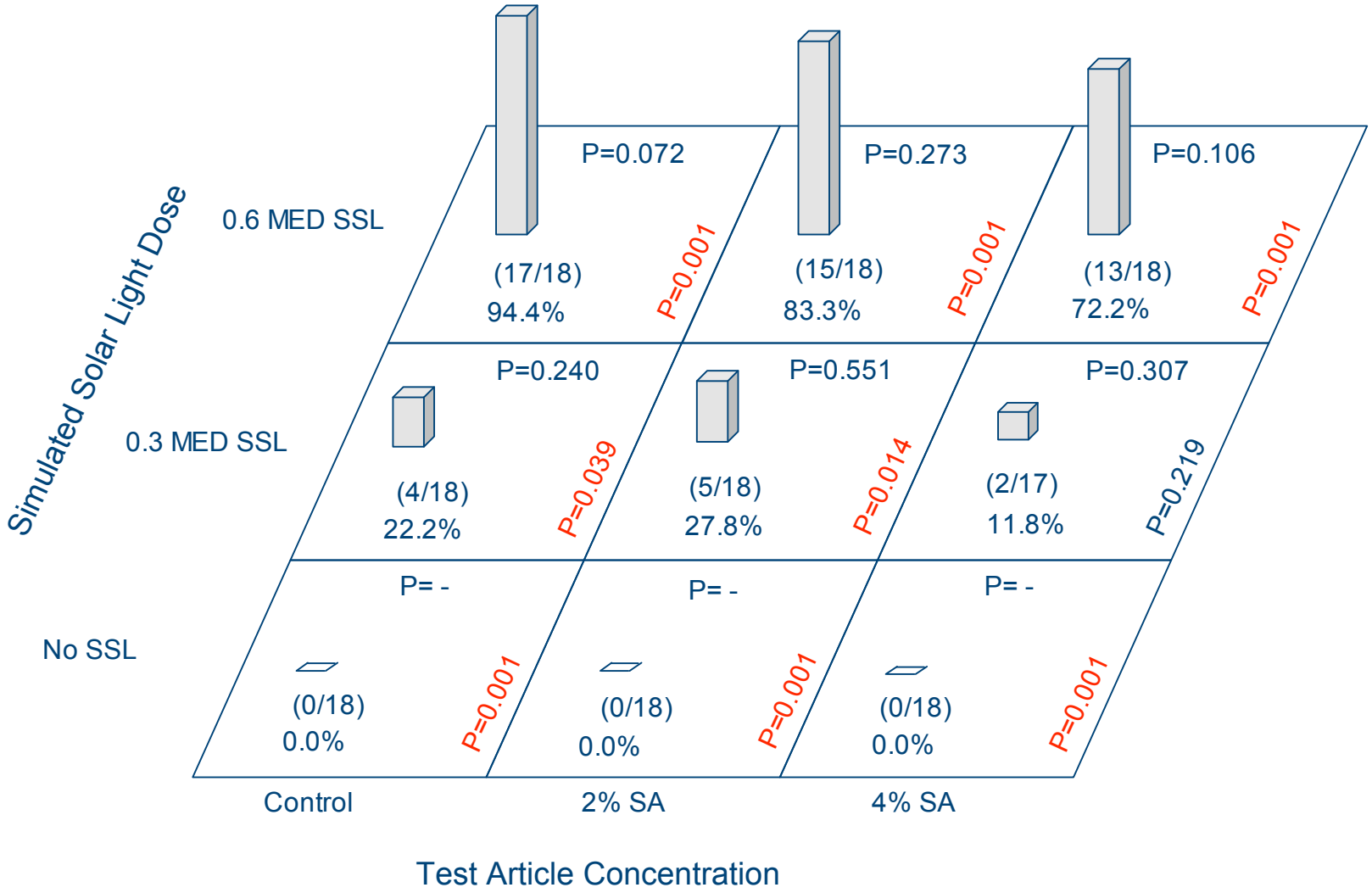
FEMALE MICE, SALICYLIC ACID; SQUAMOUS CELL CARCINOMA



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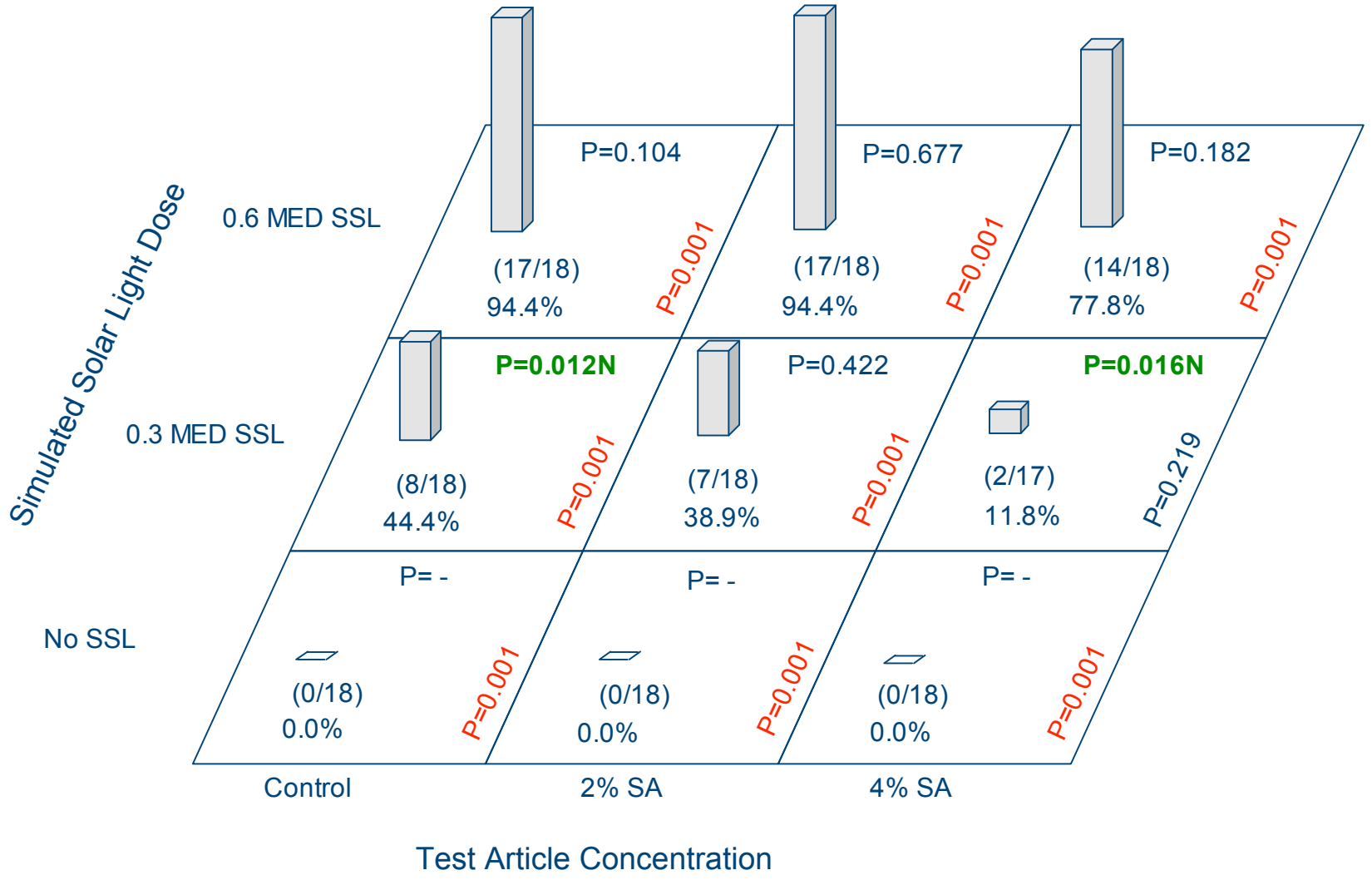
FEMALE MICE, SALICYLIC ACID; ALL SKIN CANCERS



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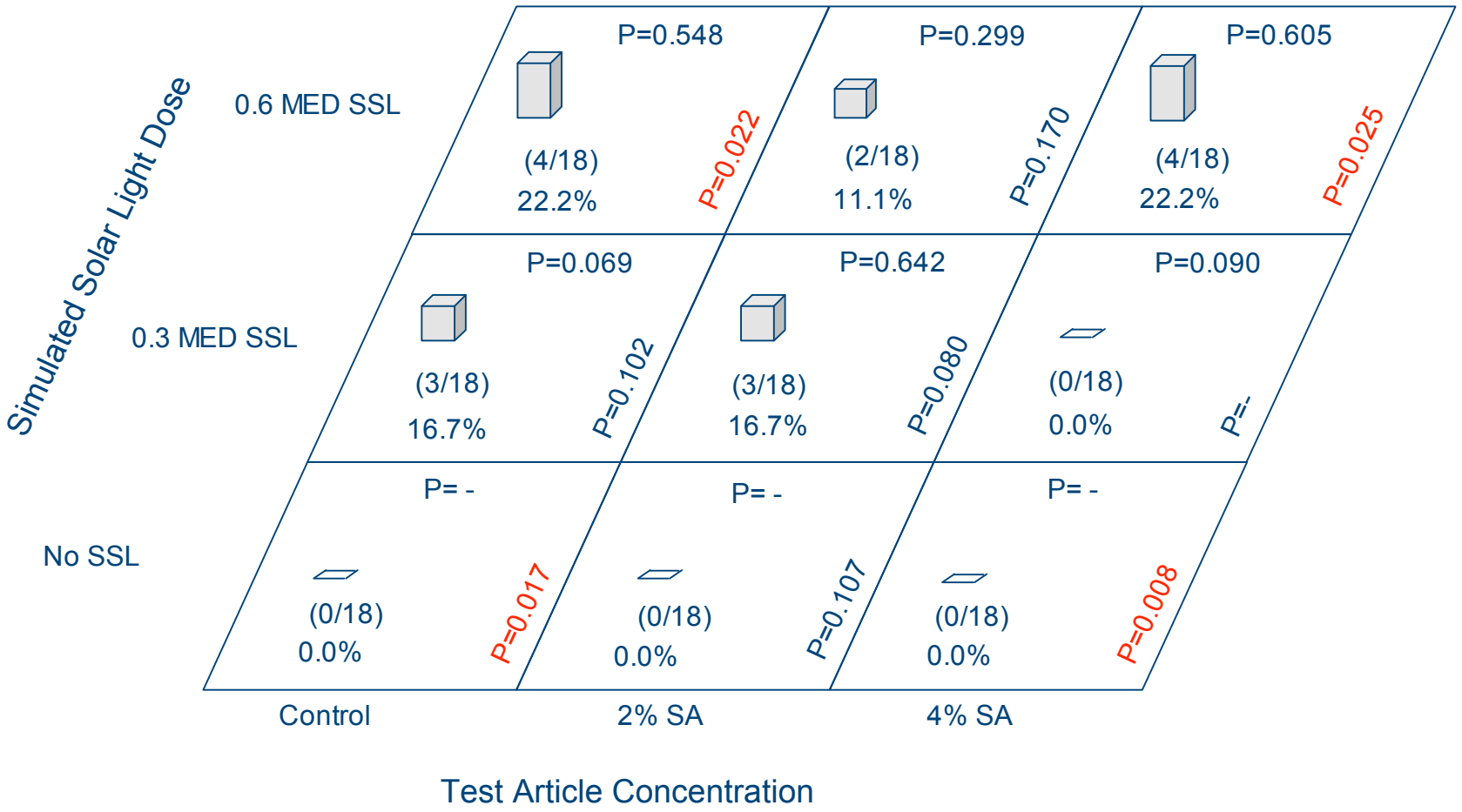
CONCLUSIONS, PATHOLOGY (3)

- Glycolic acid did not affect tumorigenesis of SSL in female mice,

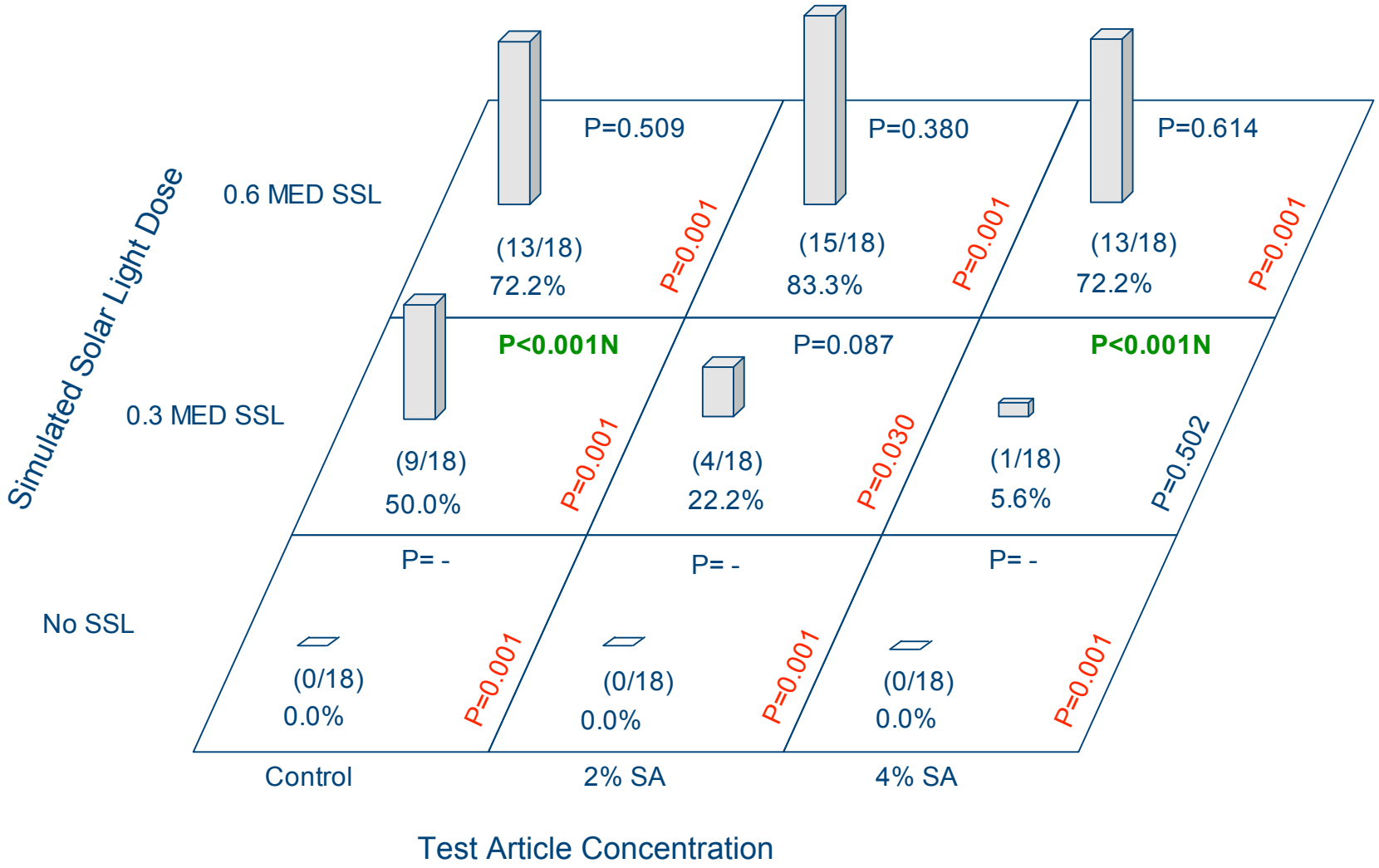
- Glycolic acid did not consistently affect tumorigenesis of SSL in male mice

- Salicylic acid was protective, reducing tumorigenesis of SSL in female mice.

MALE MICE, SALICYLIC ACID; SQUAMOUS CELL PAPILLOMA



MALE MICE, SALICYLIC ACID; CARCINOMA *IN SITU*



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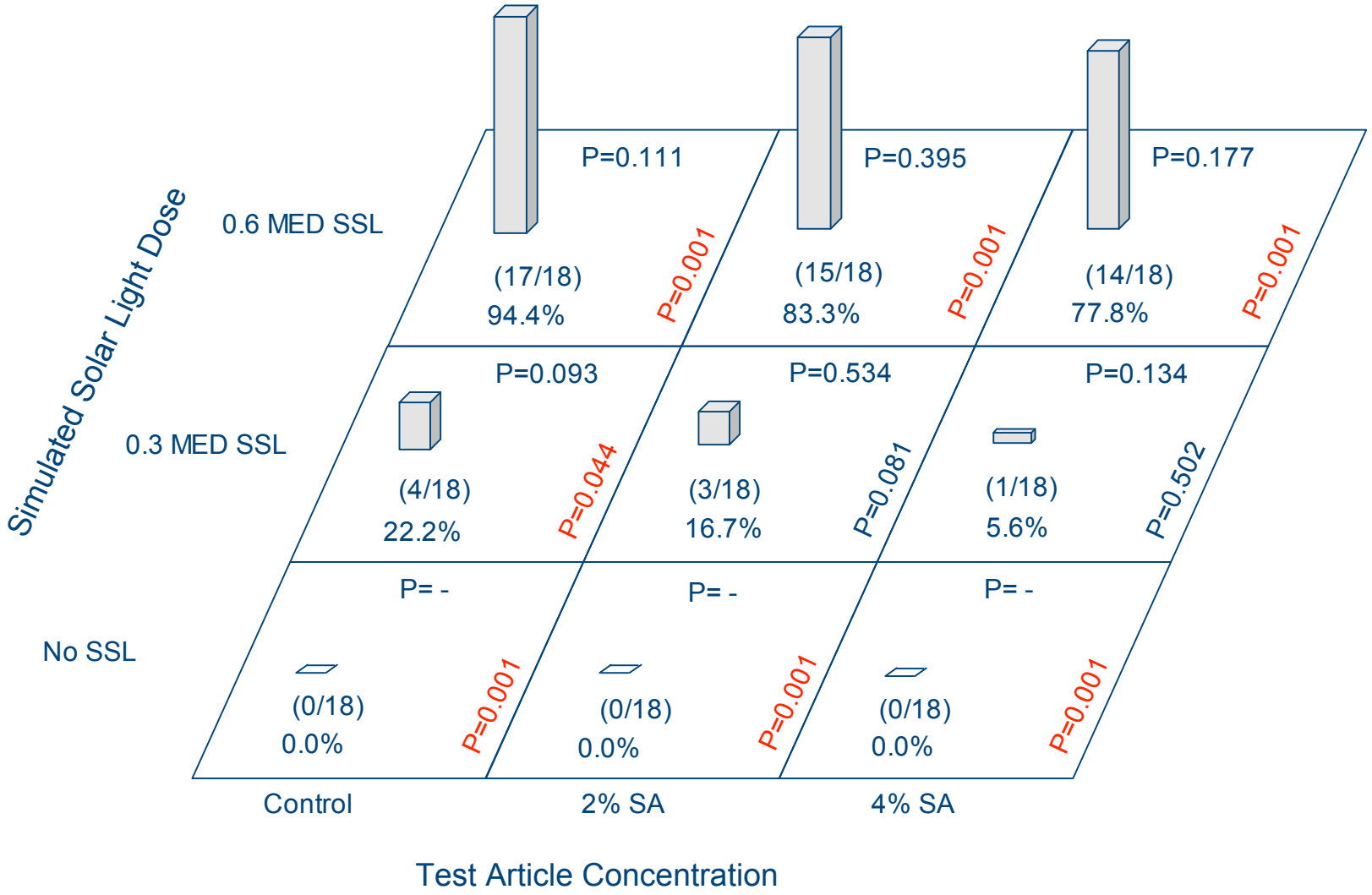
MALE MICE, SALICYLIC ACID; SQUAMOUS CELL CARCINOMA



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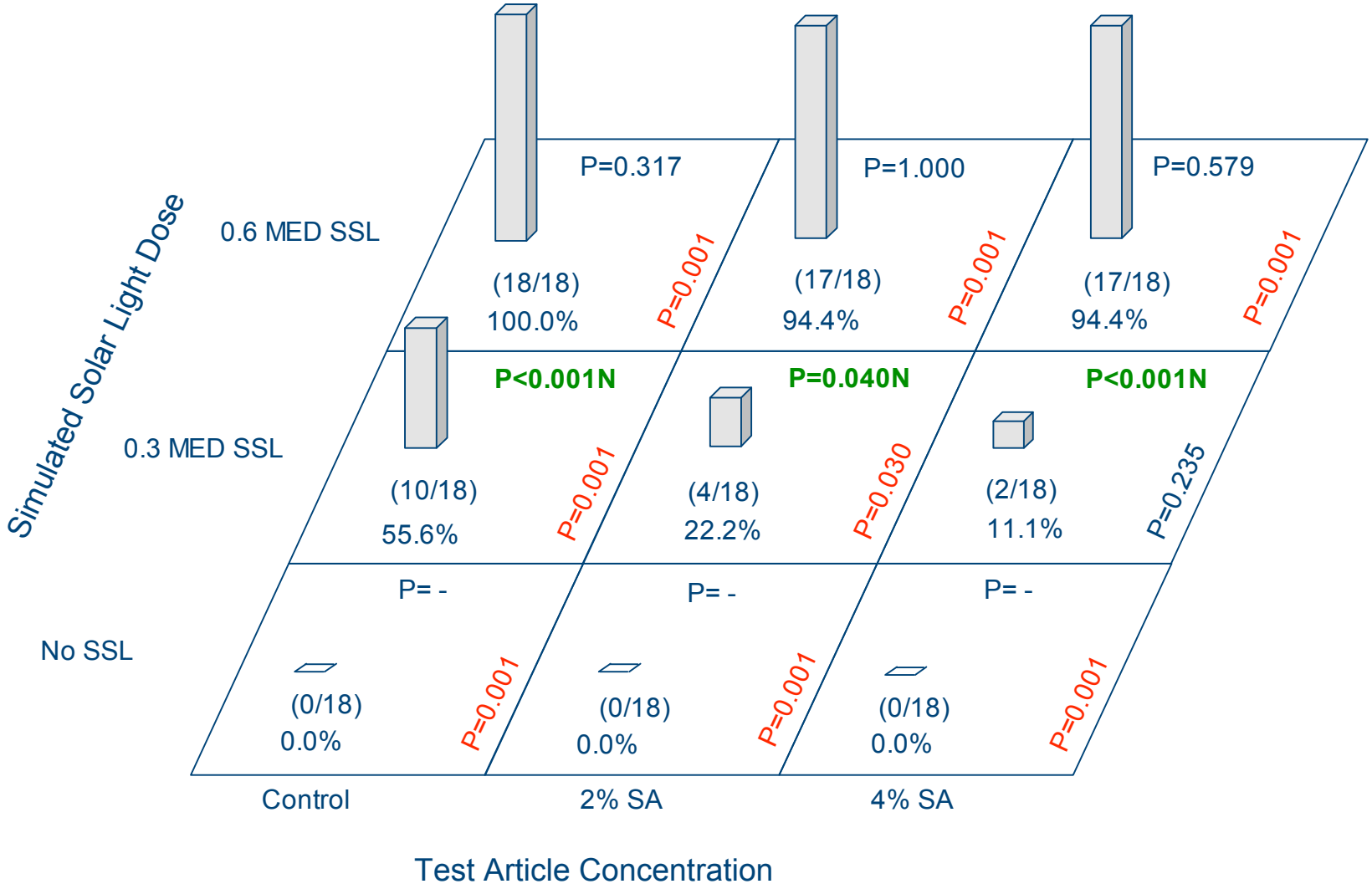

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CONCLUSIONS, PATHOLOGY (4)

- Glycolic acid did not affect tumorigenesis of SSL in female mice,
-Glycolic acid did not consistently affect tumorigenesis of SSL in male mice

- Salicylic acid was protective, reducing tumorigenesis of SSL in female and male mice.

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TUMOR MULTIPLICITY

- Tumor multiplicity statistical analyses had not been applied to photococarcinogenicity studies with SKH-1 mice.

- NCP sponsored a Tumor Multiplicity Working group to consider multiplicity modeling:

Kodel *et al*., Kokaska model

Dunson *et al*., applied to Tg.AC

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TUMOR MULTIPLICITY

The Tumor Multiplicity Working Group requested that we:

(1) Revisit modeling method, use point-process regression modeling approach. √

(a) Anderson-Gill multiplicative intensity point process regression model. √

(b) Tumor type model.

(c) Tumor growth model.

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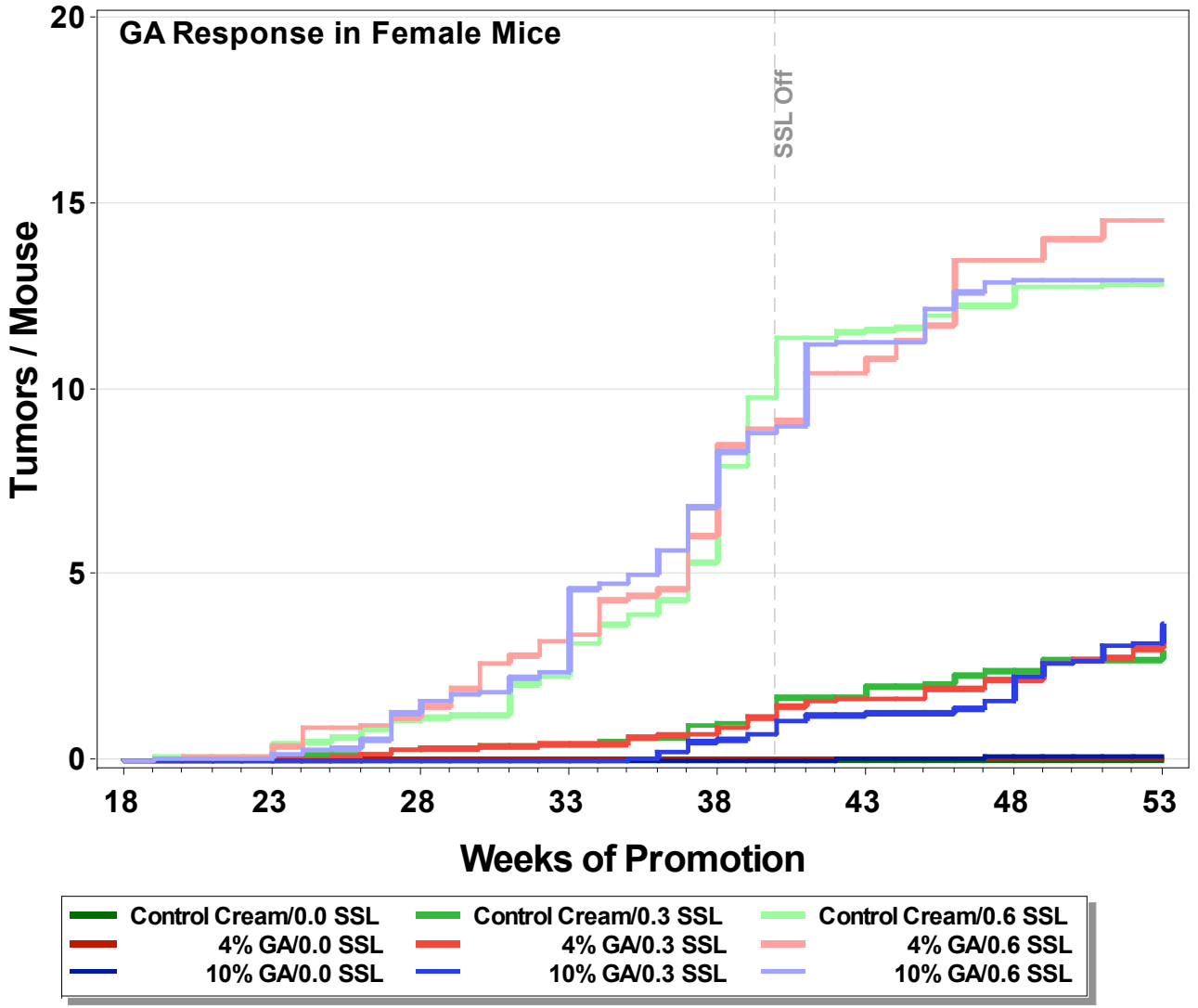
MODELING APPROACHES USED FOR TUMOR MULTIPLICITY

(i) Anderson-Gill multiplicative intensity point process regression model.

(ii) Mann-Whitney U-test (nonparametric Akritas-Arnold, essentially Kruskal-Wallis) as used in SENCAR mouse studies (LOCF; pairwise comparison at 40-week asymptote).

(iii) Wei-Lin-Weissfeld recurrent event model.

TUMOR MULTIPLICITY, FEMALE MICE, GLYCOLIC ACID



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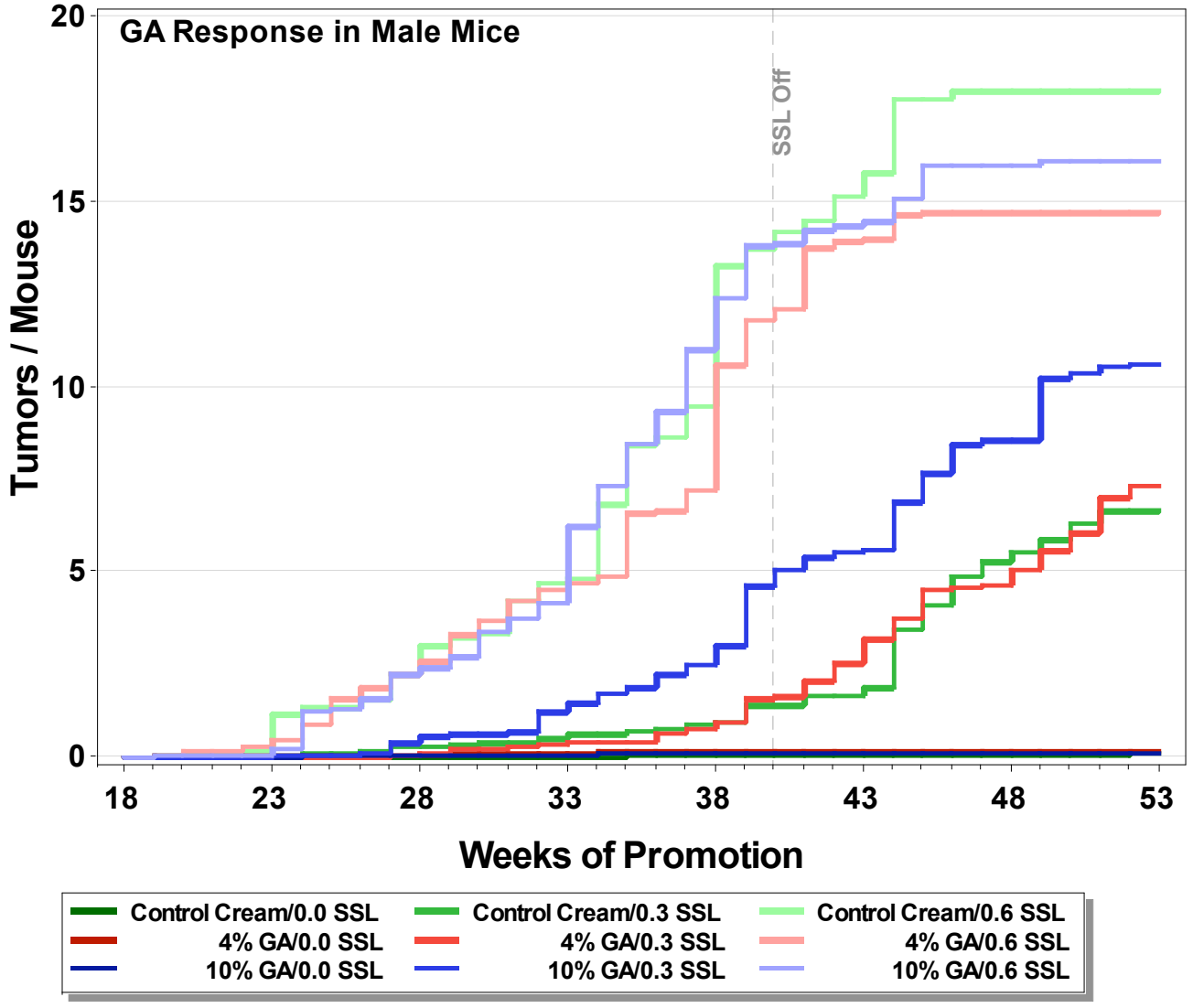
TUMOR MULTIPLICITY, MALE MICE, GLYCOLIC ACID



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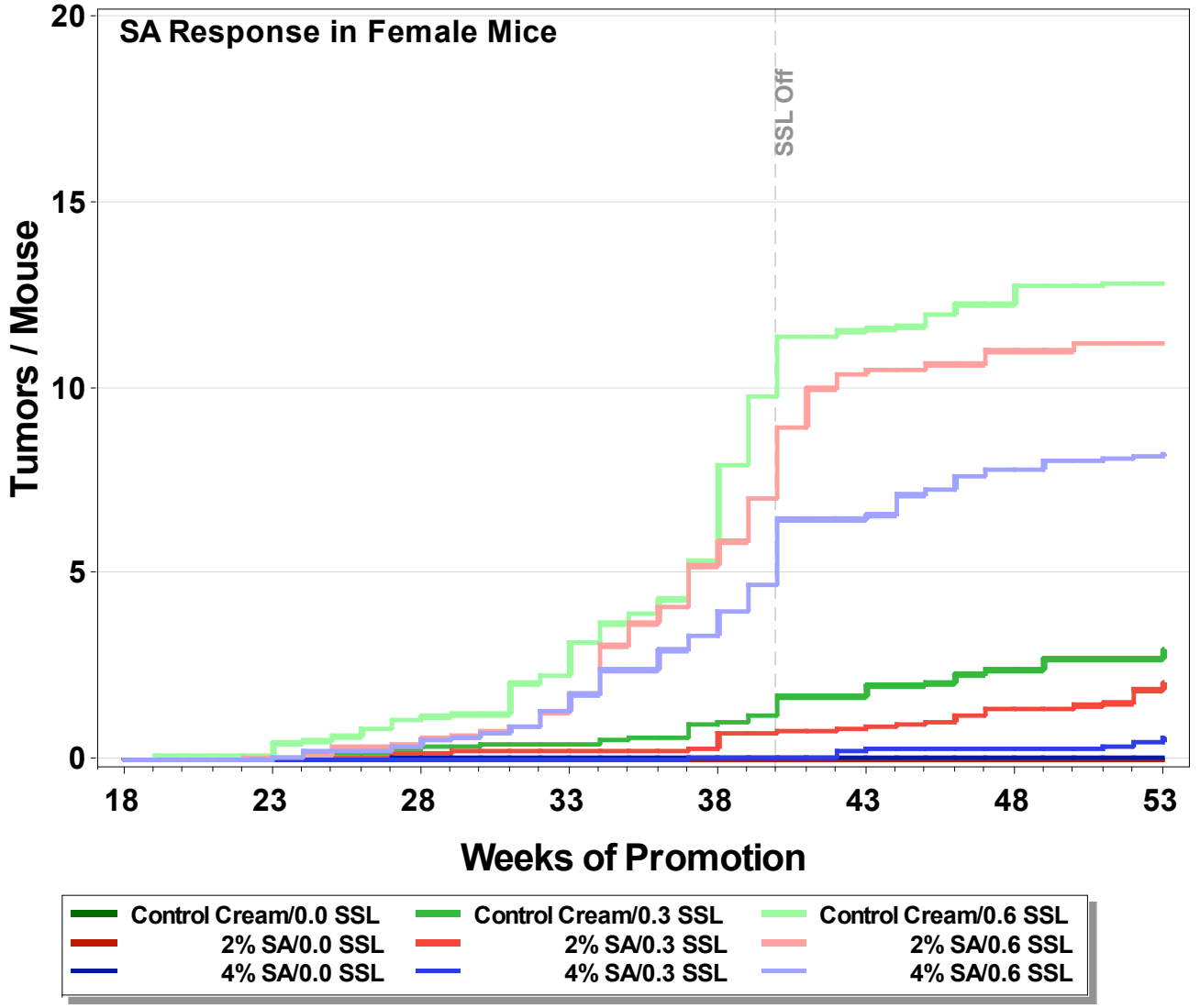
TUMOR MULTIPLICITY, FEMALE MICE, SALICYLIC ACID



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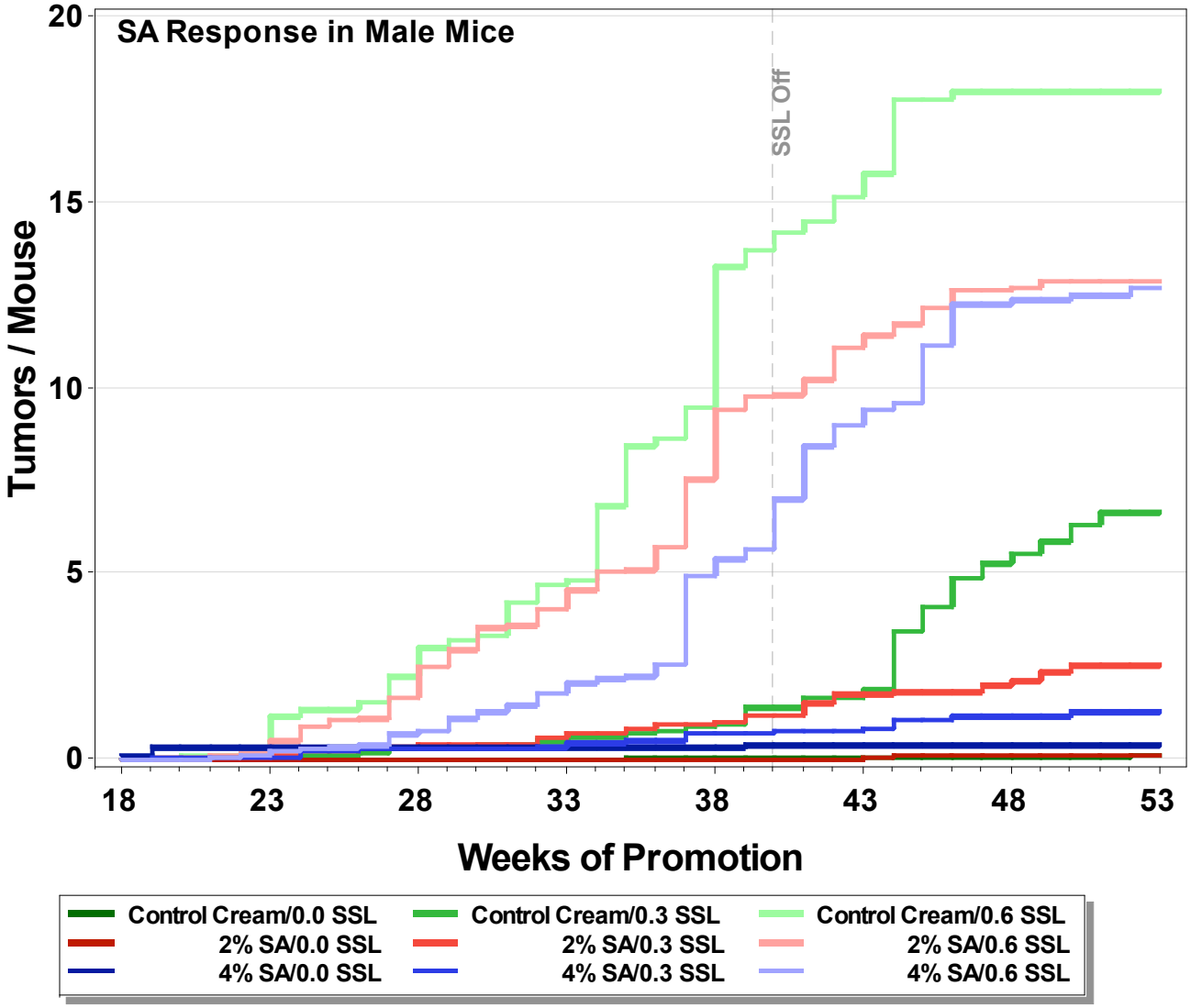
TUMOR MULTIPLICITY, MALE MICE, SALICYLIC ACID



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TUMOR MULTIPLICITY, ANDERSON-GILL METHOD

-------- Female -------- -------- Male ----------

	Vehicle	4% GA	10% GA	Vehicle	4% GA	10% GA
Relative Hazard Ratio						
0.3 MED	*100%*	108%	106%	*100%*	98%	**166%** ***p=0.042***
0.6 MED	*100%*	110%	105%	*100%*	109%	105%

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TUMOR MULTIPLICITY, ANDERSON-GILL METHOD

	-------- Female --------			-------- Male ----------		
	Vehicle	**2% SA**	**4% SA**	**Vehicle**	**2% SA**	**4% SA**
Relative Hazard Ratio						
0.3 MED	*100%*	62%	**18%** ***p=0.031***	*100%*	**37%** ***p=0.008***	**16%** ***p<0.001***
0.6 MED	*100%*	**75%** ***p<0.001***	**52%** ***p<0.001***	*100%*	**61%** ***p=0.004***	**54%** ***p=0.002***

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CONCLUSIONS, TUMOR MULTIPLICITY (1)

Only effect of glycolic acid was in male mice,

- 10% glycolic acid increased the tumor multiplicity relative risk at 0.3 MED/day.

Application of salicylic acid,

- decreased risk at 2% SA and 4% SA at both doses of SSL in males,
- decreased risk in females with 4% SA at 0.3 MED/day, and with 2% SA and 4% SA at 0.6 MED/day.

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SUMMARY CONCLUSIONS

Application of control cream,
- increased SSL effect (mean time to tumor, all skin cancers).

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SUMMARY CONCLUSIONS

The application of cream containing **glycolic acid** had an inconsistent effect:

- decreased mean time-to-tumor and increased squamous cell carcinoma only at 0.3 MED in male mice, and only with dose-trend analysis,
- tumor multiplicity was increased only in male mice at 10% GA and 0.3 MED.

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SUMMARY CONCLUSIONS

The application of cream containing **salicylic acid** was in general protective:
- at 4% SA, increased time to tumor, decreased carcinoma *in situ* and all cancers, and tumor multiplicity at 0.3 MED/day (both sexes),
- decreased multiplicity at all doses of light and salicylic acid doses (except 2% SA, 0.3 MED/day, females).

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Photocarcinogenesis Study of Glycolic Acid and Salicylic Acid in SKH-1 Mice

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TUMOR MULTIPLICITY, AKRITAS-ARNOLD METHOD

	Vehicle	4% GA	10% GA	Vehicle	2% SA	4% SA
Female						
0.3 MED	*ns*	*ns*	*ns*	***p<0.001***	*ns*	***p<0.001***
0.6 MED	*ns*	*ns*	*ns*	***p=0.006***	*ns*	***p=0.006***
Male						
0.3 MED	***p=0.006***	*ns*	***p=0.005***	*ns*	*ns*	*ns*
0.6 MED	*ns*	*ns*	*ns*	***p=0.004***	*ns*	***p=0.004***

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TUMOR MULTIPLICITY, WEI-LIN-WEISSFELD METHOD

	-------- Female --------			-------- Male ----------		
	Vehicle	**4% GA**	**10% GA**	**Vehicle**	**4% GA**	**10% GA**
Relative Hazard Ratio						
0.3 MED	*100%*	108%	106%	*100%*	97%	**174%*** ***p=0.041***
0.6 MED	*100%*	112%	110%	*100%*	115%	109%

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TUMOR MULTIPLICITY, WEI-LIN-WEISSFELD METHOD

	-------- Female --------			-------- Male ----------		
	Vehicle	**2% SA**	**4% SA**	**Vehicle**	**2% SA**	**4% SA**
Relative Hazard Ratio						
0.3 MED	*100%*	62%	**18%** ***p<0.001***	*100%*	**36%** ***p=0.007***	**16%** ***p<0.001***
0.6 MED	*100%*	74%	**48%** ***p<0.001***	*100%*	**61%** ***p=0.015***	**50%** ***p=0.002***